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CROPS, PLANT GROWTH, PLANT PRODUCTS, ACTION OF MANURES

CROPS

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Fünzig Jahre Dauerfeldversuche in der Versuchsstation Woburn, einer Abteilung der Versuchsstation in Rothamsted.

Von

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Direktor der Versuchsstation in Rothamsted.

Mit 10 Textabbildungen.

(Übersetzt von der Pflanzenzuchtstation der Universität Halle a. d. S.)

Inhalt: Einleitung. — I. Die Dauer-Gerstenversuche: 1. Das Stickstoffproblem und die „Mineralstoff-Theorie“. 2. Die Wirkung der Mineraldüngungen. 3. Die Wirkung stickstoffhaltiger Düngemittel. 4. Die Wirkung von Kalk auf saurem Boden. 6. Rapskuchen als Düngung. 7. Die den Pflanzen zugeführte Stickstoffmenge. 8. Die Nachwirkung stickstoffhaltiger Düngemittel: a) schwefelsaures Ammoniak und Natronsalpeter; b) Stallmist. 9. Korn- und Strohausbildung. 10. Die jährlichen Ertragsschwankungen 11. Witterungs- und andere Jahreseinflüsse. — II. Die Weizen-Versuche: 1. Die ungedüngten Parzellen. 2. Mineralsalzdüngungen ohne Stickstoff. 3. Mineralsalzdüngungen. 4. Stickstoffhaltige Düngemittel (schwefelsaures Ammoniak und Natronsalpeter) und ihre Nachwirkung. 5. Stallmist und seine Nachwirkung. 6. Rapskuchen. 7. Verhältnis von Kornertrag zu Gesamtertrag. 8. Jährliche Ertragsschwankungen bei Weizen. 9. Einfluß der Niederschläge auf Weizenernten. — III. Die Möglichkeit, den Ausfall der Getreideernten vorauszusagen: 1. Die Zusammensetzung des Getreidekorns. 2. Bodenverschlechterung bei ständigem Getreidebau. — IV. Fruchtwechselversuche: Nachwirkung des Düngers bei Verfütterung von Baumwollkuchen und Mais.

Einleitung.

Die Versuchsstation Woburn wurde im Jahre 1876 von dem Herzog von Bedford und der Königlichen Landwirtschafts-Gesellschaft zu dem Zweck gegründet, bestimmte Versuche durchzuführen, die sich sonst nicht ermöglichen ließen.

Die Versuchsstation Rothamsted, deren Gründung bereits mehr als 30 Jahre zurücklag, war damals die einzige ihrer Art in Großbritannien und gehörte Sir John Lawes, der sie 1843 gegründet hatte. Die Station Woburn erfreute sich der ungeteilten Sympathie und Unterstützung sowohl von Lawes als auch von Gilbert, und viele Jahre lang nahmen sie regen Anteil und erteilten hinsichtlich Problemstellung und Ergebnisse ihre Ratschläge.

Eines der Hauptprobleme, die gelöst werden sollten, war, Beobachtungen darüber zu machen, in welchem Grade die Bodenfruchtbarkeit abhängig ist von dem Verfüttern hochwertiger Futtermittel (wie Ölkuchen und Getreide) an das Vieh. Diese Futtermittel bereicherten unverkennbar die Düngung, die ihrerseits die Fruchtbarkeit des Bodens steigern konnte. Nach dem landwirtschaftlichen Pachtgesetz von 1815 hatte ein Pächter, der sein Gut aufgab, Anspruch auf Entschädigung nach dem Wert der

Verbesserungen, die er dem Besitz während seiner Pachtzeit hatte zukommen lassen. Unter diese Verbesserungen fiel auch die Erhöhung der Fruchtbarkeit, die nach dem Verbrauch des Kraftfutters bemessen wurde.

Zwar setzte das Gesetz genau fest, wofür Entschädigung gezahlt werden mußte, aber es brachte darüber keine Angaben, wie die Vergütung berechnet werden sollte. Damals waren noch keine Versuche in dieser Richtung durchgeführt worden und die Station wurde deshalb ins Leben gerufen, um diese notwendigen Untersuchungen vorzunehmen.

Es soll gleich vorausgeschickt werden, daß dieser Teil des Programms noch nicht zum Abschluß gekommen ist. Selbst nach 60jähriger Arbeit

Tabelle 1.
Die Böden in Rothamsted und Woburn.

Im Trockenschrank getrocknete Proben in Prozenten des lufttrockenen Bodens

	Weizenparzellen in Rothamsted		Weizenparzellen in Woburn	
	Parzelle 2	Parzelle 10	Parzelle 11 b	Parzelle 2 a
	Düngung			
	Stallmist	Schwefel- saures Ammoniak allein	Stallmist	Schwefel- saures Ammoniak allein
Grober Sand (2–0,2 mm) . .	6,1	5,6	50,0	61,2
Feiner Sand (0,2–0,02 mm) . .	42,5	46,1	24,3	20,0
Schluff (Silt) (0,02–0,002 mm) .	24,5	26,2	8,5	6,0
Rohton oder Kolloidton (unter 0,002 mm)	18,2	20,0	13,3	10,3
Kohlensäure Salze	1,9	1,6	—	—
Feuchtigkeit des lufttrockenen Bodens . .	3,9	2,7	1,3	0,9
Verluste durch Lösung	1,4	0,4	0,9	0,6
Unterschied	1,5	— 2,9	1,7	1,0
Summe	100,0	100,0	100,0	100,0
Verluste durch Verbrennung	8,5	5,5	3,9	3,0

bleibt es ungewiß, ob überhaupt für die Entschädigung feste Richtlinien aufgestellt werden können; im allgemeinen beurteilen Taxatoren die Fruchtbarkeit des Bodens mehr nach der Ernte als nach dem, was der Bauer dem Boden zugeführt hat, obgleich es bekannt ist, daß dieses wie alle anderen wichtigen Faktoren ebenfalls berücksichtigt werden muß.

Zu derselben Zeit wurden Versuchsserien mit ununterbrochenem Weizen- und Gerstenbau begonnen; diese Versuche führte man nach ähnlichen Richtlinien wie in Rothamsted durch. Man wollte ermitteln, wie weit die in Rothamsted auf schwerem Lehm Boden gewonnenen Ergebnisse sich in Woburn auf leichtem Sandboden wiederholen ließen. Tabelle 1 zeigt den Unterschied zwischen den Böden durch Gegenüberstellung der Resultate bei der mechanischen Analyse der beiden Böden. Es ist günstig, daß die Unterschiede in den klimatischen Verhältnissen der beiden Stationen

nur gering sind. Angaben darüber gibt Tabelle 2. Woburn hat eine geringere Niederschlagsmenge als Rothamsted und eine etwas höhere Durchschnittstemperatur; aber diese Unterschiede sind ziemlich gut über das ganze Jahr verteilt.

Die Ergebnisse von Woburn stimmen in vielen Punkten mit denjenigen von Rothamsted überein. Wenn die Unterschiede geringer sind als die auf den jeweiligen Stationen in verschiedenen Jahren erhaltenen, so zeigt das, daß die Wirkung von Wetter und anderen klimatischen Bedingungen auf den Ertrag größer ist als die Einwirkung des Bodens. In Woburn zeigte sich jedoch eine auffallende Verschlechterung des Bodens bei der Anwendung von schwefelsaurem Ammoniak, das hier eine Ver-

Tabelle 2.
Durchschnittliche monatliche Niederschlagsmenge und Temperatur
in Woburn und Rothamsted.

	Niederschlagsmenge		Temperatur	
	Woburn 1877 bis 1931	Rothamsted 1877 bis 1931	Woburn 1896 bis 1917	Rothamsted 1878 bis 1931
	mm	mm	° C	° C
September	49,25	59,50	13,3	13,2
Oktober	64,00	78,50	9,5	9,2
November	55,50	72,00	5,8	5,7
Dezember	55,00	71,75	4,0	3,6
Januar	44,75	60,25	3,4	3,1
Februar	38,75	50,75	3,7	3,6
März	39,75	49,25	5,1	5,1
April	43,50	51,25	7,7	7,4
Mai	48,50	51,75	11,3	11,1
Juni	48,25	55,25	14,3	13,9
Juli	61,00	68,50	16,4	15,8
August	60,00	66,50	15,9	15,4
Durchschnittliche Jahresmenge	608,25	735,25		
Durchschnittliche Jahrestemperatur			9,2	8,9

sauerung des Bodens hervorrief, eine Wirkung, die bis jetzt in Rothamsted nicht beobachtet wurde.

Eine dritte wichtige Versuchsreihe wurde mit Gründung durchgeführt. Diese Versuche sollten besonders den Wert von Wicke (*Vicia*) und Senf (*Sinapis*) als Vorfrucht vor Weizen feststellen. Trotz beträchtlicher Gründungsgaben wurde jedoch die Ernte nicht wesentlich gesteigert.

Von 1876 bis zu seinem Tode im Jahre 1884 hatte Dr. Augustus Voelcker die Leitung dieser Versuche in der Hand. Sie wurde dann von seinem Sohn Dr. J. A. Voelcker übernommen. Im Oktober 1921 gab die Königliche Landwirtschafts-Gesellschaft den Betrieb auf; aber Dr. Voelcker führte ihn privat weiter, bis er im Oktober 1926 von der Station Rothamsted übernommen wurde. Jetzt ist die Station Woburn als Unterabteilung der Station Rothamsted angeschlossen und untersteht demselben Komitee und der gleichen Verwaltung. Kürzlich wiederum hat

die Königliche Landwirtschafts-Gesellschaft der Station Rothamsted für mehrere Jahre einen besonderen Zuschuß bewilligt, der es ermöglichte, die Ergebnisse in der Statistischen Abteilung genau durchprüfen zu lassen. Ein vollständiger Bericht ist als eine der Monographien von Rothamsted unter dem Titel „50 Jahre Feldversuche der Versuchsstation Woburn“ erschienen; die wichtigsten Ergebnisse, die von allgemeinem Interesse sein dürften, sind in der vorliegenden Arbeit zusammengefaßt.

I. Die Dauer-Gerstenversuche.

1. Das Stickstoffproblem und die „Mineralstoff-Theorie“.

Der Versuchsplan, nach dem in den ersten fünf Jahren gearbeitet wurde, zeigt, daß die Absicht der Versuchsansteller darauf hinausging, weitere Studien über die damals als „Stickstoff-Problem“ bekannte Fragestellung zu machen: Nämlich die Frage, ob Pflanzen Stickstoffgaben brauchen oder ob — wie Liebig lange vorher behauptet hatte — sie sich allein mit mineralischen Düngemitteln entwickeln können wie Phosphaten, Alkalisalzen und alkalischen Erden, Kalium, Natrium, Magnesium und Kalzium. Die Auseinandersetzung über diese Frage war eine der wichtigsten Aufgaben in Gilberts Leben und obgleich die meisten glaubten, daß die Versuche in Rothamsted die Notwendigkeit der Stickstoffdüngung außer jeder Frage gestellt hatten, wurden weder Gilbert noch Lawes gern auf die Möglichkeit verzichtet haben, das Problem weiter zu erforschen.

Überdies waren im Verlaufe der Versuche in Rothamsted und ihrer Anwendungen in der Praxis eine Anzahl von Problemen aufgetaucht, die es notwendig machten, Untersuchungen auf Böden durchzuführen, die von dem in Rothamsted abwichen.

Folgende Fragen wurden bearbeitet.

1. Kann Gerste bei leichtem Boden Jahr für Jahr auf demselben Land gedeihen?
2. Kann Mineraldüngung allein die Fruchtbarkeit erhalten oder ist auch eine Stickstoffdüngung notwendig?
3. Kann Stickstoffdüngung allein die Fruchtbarkeit erhalten oder ist auch Mineraldüngung erforderlich?
4. Angenommen, Stickstoffdüngung erweist sich als notwendig, wie läßt sich dann schwefelsaures Ammoniak als Stickstoffquelle mit Natronsalpeter vergleichen?
5. Wie stellt sich Stallmistdüngung zu künstlicher Düngung?
6. Wie verhält sich eine doppelte Menge Stickstoff entweder als Stallmist oder als Kunstdünger im Vergleich zu einer einfachen Gabe?

Die Versuche wurden in allen Parzellen nach dem damals für richtig gehaltenen folgenden einfachen Schema angestellt:

1. Ungedüngt,
2. Mineraldüngung allein, kein Stickstoff.

3. Keine Mineraldüngung, nur Stickstoffgaben:

- a) Ammoniumsalze,
- b) Natronsalpeter,

4. Mineral- + Stickstoffgaben:

- a) Ammoniumsalze,
- b) Natronsalpeter,

5. wie 4. aber Stickstoffgaben in doppelter Menge.**6. Stallmistgabe in einfacher und doppelter Menge.**

Die Stickstoffmengen, die in den künstlichen Düngemitteln gegeben wurden, betrugen 46 und 92 kg je Hektar; das ist etwas weniger als in Rothamsted, wo 48 kg je Hektar die Norm ist. Bei der Stallmistdüngung wurden die Mengen nach der Zusammensetzung des vorher den Tieren verabreichten Futters berechnet unter Berücksichtigung des Stickstoffes, den die Tiere entsprechend ihrer Lebendgewichtszunahme aufgenommen hatten. Die Zahlen, die auf diese Weise erhalten und in den Berichten als „100 $\%$ NH_3 “ und „200 $\%$ NH_3 “ je acre (1 acre = 4046.7838 qm) bezeichnet worden sind, entsprechen 92,1 bzw. 184 kg Stickstoff je Hektar. Man nahm dabei an, daß kein Stickstoffverlust eingetreten war. Später jedoch hat Dr. J. A. Voelcker in den drei Jahren 1899 bis 1901 sorgfältige Beobachtungen über diesen Prozeß gemacht und gezeigt, daß der Verlust annähernd 32% betrug, so daß die für den Boden verwendeten wirksamen Mengen sicher nicht die oben genannte Höhe erreichten, sondern wahrscheinlich ungefähr 62 und 123 kg je Hektar betrugen. Diese Zahlen sind in der vorliegenden Arbeit zugrunde gelegt.

Die durchschnittlichen Ergebnisse der in den ersten fünf Jahren durchgeführten Versuche sind in Tabelle 3 gegeben. Die Erträge sind hoch, besonders auf der ungedüngten Parzelle. Sie zeigen keine Anzeichen von irgendwelchem Sinken des Ertrages, obgleich der Boden für arm gehalten wurde. Mineralsalzgaben allein bewirkten keine weitere Steigerung des Ertrages, stickstoffhaltige Düngung allein ergab eine deutliche Zunahme des Ertrages, während eine Volldüngung noch höhere Ernten erbrachte.

Gegenüber der Ernte von einer Parzelle ohne Stickstoffgabe vermehrte die erste Stickstoffgabe die Ernte um 2648 kg je Hektar im Gesamtertrag und 1120 kg je Hektar im Kornertrag. Jedoch zeigte die zweite Stickstoffgabe eine geringere Wirkung: sie brachte nur eine Zunahme von 1120 kg je Hektar Gesamtertrag und 336 kg je Hektar Kornertrag. Stallmist hatte eine geringere Wirkung als künstlicher Dünger, selbst die stärkere Düngung (123 kg Stickstoff je Hektar) ergab eine geringere Ernte als die Volldüngung, bei welcher nur 46 kg Stickstoff je Hektar gegeben wurde. Durch eine einfache Interpolation können wir schließen, daß 123 kg Stickstoff je Hektar, im Stallmist gegeben, einer Menge von nur 28 kg Stickstoff im Natronsalpeter oder im schwefelsauren Ammoniak je Hektar entspricht.

Zwischen schwefelsaurem Ammoniak und Natronsalpeter bestand fast kein Unterschied in der Wirkung.

Tabelle 3.

Die Gersten-Erträge der Jahre 1877 bis 1881 in Doppelzentnern je Hektar.

Parzelle	Düngung je Hektar	Stickstoff im Düngemittel kg/ha	Woburn			Rothamsted		
			Gesamtertrag	Korn-ertrag	Korn vom Gesamt-ertrag %	Gesamtertrag	Korn-ertrag	Korn vom Gesamt-ertrag %
1	Ungedüngt			15,2 12,1	42 13,7	17,1	8,6	50
7	Ungedüngt		31,1		42			
4	Mineraldüngung ¹⁾		28,9	12,6	43	21,6	11,0	51
2	Ammoniumsälze	16 ²⁾	48,2	21,2	43	33,2	16,8	50,5
3	Natronsälpeter	46	48,0	20,0	41	36,0	17,4	48,5
5	Wie 4 + Ammoniumsälze	46	55,0	23,3	42	54,4	26,2	48
6	Wie 4 + Natronsälpeter	46	57,3	23,8	41	56,9	26,8	47
8	Wie 4 + Ammoniumsälze	92	66,6	27,6	41			
9	Wie 4 + Natronsälpeter	92	68,6	27,0	39			
10	Stallmist	62 ³⁾	36,8	15,8	42			
11	Stallmist	123 ⁴⁾	46,7	21,1	45	69,1	32,0	46

Die Ergebnisse sind im allgemeinen denjenigen ähnlich, die man in Rothamsted erhalten hat. Der Hauptunterschied zwischen den ungedüngten Parzellen und denjenigen mit künstlichen Düngemitteln zeigt sich deutlich in dem Verhältnis von Korn- zum Gesamtertrag: In Rothamsted bestand 50 % des Gesamtertrages aus Korn, während in Woburn der Anteil nur 42 % betrug; an beiden Orten gaben Natronsälpeter einen etwas niedrigeren Anteil als schwefelsaures Ammoniak. Aber merkwürdigerweise erzielte man an beiden Orten mit Stallmist denselben Anteil (45 bis 46 %).

Spätere Untersuchungen ergaben zwei weitere Unterschiede zwischen Rothamsted und Woburn: in Rothamsted fiel der Ertrag erheblich bei Weglassung von Phosphordüngung, während man in Woburn eine beträchtliche Verminderung nicht beobachten konnte. Noch auffällender war es, daß die dauernde Anwendung von schwefelsaurem Ammoniak den Boden in Woburn sauer machte, während in Rothamsted, wo mehr Kalziumkarbonat vorhanden war, Säure nicht festgestellt werden konnte. Eine kleine Änderung in der Versuchsanstellung wurde vor 1881 vorgenommen, und nach 1906 wurden die Düngergaben bedeutend herabgesetzt. Die Menge der Düngemittel und die Erträge sind in Tabelle 3 wiedergegeben. Den Versuchsplan zeigt Abbildung 1.

Ungedüngte Parzellen. Die Erträge der beiden ungedüngten Parzellen während der ersten neun Jahre sind in Tabelle 4 dargestellt.

¹⁾ 4,1 dz Superphosphat, 2,21 dz Kalisulfat, 1,12 dz Natriumsulfat, 1,12 dz Magnesiumsulfat.

²⁾ 16 kg in Woburn und 48 kg in Rothamsted.

³⁾ Schätzungsweise. Die in den Berichten aufgezeichneten Mengen sind „1,12 dz NH₃“, bzw. „2,24 dz NH₃“.

⁴⁾ Ungefähr 2,24 dz in Rothamsted.

Anordnung der Parzellen beim Dauergerstenversuch.

(Stackyard — Feld)

10 A Superphosphat 3,77 dz Natronsalpeter = 23 kg N	10 B Rapskuchen = 92 kg N	3 BB Natronsalpeter = 46 kg N mit 50 dz Kalk im Januar 1921	3 AA Natronsalpeter = 92 kg N mit 50 dz Kalk im Januar 1921	2 BB Schwefelsaures Ammoniak 46 kg N mit 50 dz Kalk im Dezember 1897 und Mai 1905	2 AA Schwefelsaures Ammoniak 46 kg N mit 6,25 dz Kalk, Mai 1905 und noch- mals 1909, 1910, 1912	1 Ungeädert
11 A Schwefelsaures Kali 1,25 dz Natronsalpeter = 23 kg N	11 B Stallmist-Düngung 123 kg N	6 Mineralsalzdüngungen, und Natronsalpeter 46 kg N		5 B Wie 5 A aber mit 50 dz Kalk Dezember 1897 und 1912		1 B Mineralsalz- düngungen mit 25 dz Kalk März 1905
12 A Wie 8 B Schwefelsaures Ammoniak = 92 kg N 1921 zugeführt	12 B Wie 8 B Schwefelsaures Ammoniak = 92 kg N 1921 zugeführt	9 B Mineralsalzdüngungen und ein Jahr um das andere Natronsalpeter = 92 kg N 1921 fortgelassen	9 A Wie 9 B Natronsalpeter = 92 kg N 1921 zugeführt	8 BB Mineralsalzdüngungen und ein Jahr um das andere schwefelsaures Ammoniak 92 kg N 1921 fortgelassen	8 AA 50 dz Kalk Dezember 1897 und 1912	
		7 Ungeädert		4 A Nat Mineralsalz- düngungen		

Abb. 1.

Versuchsjahr 1921: Bei Parzelle 8 A, 8 AA und 9 A stickstoffhaltiges Düngemittel zugeführt,
bei 8 B, 8 BB und 9 B fortgelassen

Tabelle 4.
Kornertrag in Doppelzentnern je Hektar.

Parzelle	1877	1878	1879	1880	1881	1882	1883	1884	1885
1	18,5	13,7	10,8	19,1	18,8	19,3	17,9	19,6	12,8
7	12,2	10,8	7,4	12,2	18,1	15,5	14,8	19,7	12,5

Über einige dieser guten Ernten waren die Versuchsansteller ziemlich überrascht. Das Land befand sich nicht in außergewöhnlich gutem Zustande, denn in dem Jahre, bevor der Versuch angesetzt wurde, 1876, hatte der Weizen auf diesem Boden nur einen Ertrag von 23,0 hl je Hektar gebracht mit einem Strohertrag von 25,7 dz je Hektar. Der Gerstenерtrag des Jahres 1877 betrug durchschnittlich 18,9 hl je Hektar, was also einen ganz normalen Ertrag bedeutet. Dann aber nahmen nach dem schlechten Sommer von 1879 die Erträge plötzlich zu und gelangten auf Parzelle 1 zu einer unerwarteten Höhe von 29,7 hl je Hektar; auf diesem Punkt hielten sich die Erträge fünf Jahre hindurch. Damals waren einige gute Jahre; 1882, 1883 und 1884 sind alle über dem Durchschnitt gewesen. Aber eine Ernte von dieser Höhe erfordert eine Stickstoff-Assimilation von ungefähr 45 kg je Hektar, eine Summe, die für einen acht oder mehr Jahre hindurch ungedüngten Sandboden ganz außergewöhnlich erscheint. Jedoch nahmen die Erträge nach 1884 auf beiden Parzellen gleichmäßig ab.

2. Die Wirkung der Mineräldüngungen.

Phosphate und Kali.

Einige Jahre hindurch hatten Mineräldüngungen (Superphosphat, Sulfate von Kali, Natrium, Magnesium) — wenn allein gebraucht — keinen Einfluß auf die Getreideernten: als jedoch die Verschlechterung einsetzte, verlangsamten sie das Absinken. In Tabelle 5 sind die Erträge in Doppelzentner je Hektar gegeben.

Tabelle 5.

Parzellen	Erste 15 Jahre 1877 bis 1891		Zweite 15 Jahre 1892 bis 1906		Letzte 20 Jahre 1907 bis 1926	
	Gesamt- Ertrag	Korn- Ertrag	Gesamt- Ertrag	Korn- Ertrag	Gesamt- Ertrag	Korn- Ertrag
Mineräldüngung (4) . .	29,4	13,4	22,1	10,5	17,1	6,7
Ungedüngt (1 und 7) . .	30,0	13,4	17,7	8,0	13,5	5,0
Unterschied	—	—	4,4	2,5	3,6	1,7

Die Mineräldüngung wurde nach dem Jahre 1906 erheblich vermindert.

Einen großen Einfluß auf das Steigen der Getreideernten übten die Mineralsalze nur dann aus, wenn sie als Zusatz zu Natronsalpeter oder schwefelsaurem Ammoniak gegeben wurden, und dann war ihre Wirkung

in den zweiten 15 Jahren sogar noch auffallender als in den ersten 15 Jahren. Die Erträge, Doppelzentner je Hektar, sind in Tabelle 6 aufgezeichnet.

Tabelle 6.

	Parzelle	Erste 15 Jahre 1877 bis 1891		Zweite 15 Jahre 1892 bis 1906		Letzte 20 Jahre 1907 bis 1926			
		Gesamt- ertrag	Korn- ertrag	Gesamt- ertrag	Korn- ertrag	Gesamtertrag		Kornertrag	
Natronsalpeter									
Mineralsalze	6 ¹⁾	62,9	26,9	49,1	21,7	27,7		10,8	
Keine Mineralsalze	3	51,1	22,0	33,6	15,0	17,7		6,7	
Zunahme		11,8	4,9	15,5	6,7	9,0		4,1	
						Mit Kalk	Ohne Kalk	Mit Kalk	Ohne Kalk
Schwefelsaures Ammoniak									
Mineralsalze	5 ²⁾	56,0	25,2	20,2	8,9	26,4	9,1	10,6	3,0
Keine Mineralsalze	2 ³⁾	49,0	22,1	13,2	5,6	18,1	3,0	6,9	1,1
Zunahme		7,0	3,1	7,0	3,3	8,3	6,1	3,7	1,9

Ursprünglich hatte man nicht beabsichtigt, einen Versuch durchzuführen, um die Wirkung von Kali und Phosphorsäure miteinander zu vergleichen. Später wurden für diesen Zweck noch Parzellen hinzugenommen. Doch geben sie kein klares Bild, abgesehen davon, daß keines der beiden Düngemittel besonders oder ausschließlich nötig zu sein schien.

Viel später in Woburn durchgeführte Versuche haben gezeigt, daß nur gelegentlich entweder Kali oder Phosphorsäure eine deutliche Ertragssteigerung bei Gerste oder Weizen hervorruft.

Die später (S. 234) aufgezeichneten Bestimmungen des Stickstoffgehaltes des Kornes zeigen, daß sich die Wirkung der Mineralsalze in zwei Richtungen erstreckt: Sie steigern einmal die gesamte Stickstoffaufnahme der Pflanzen vermutlich dadurch, daß sie eine bessere Entwicklung der Wurzel gewährleisten und zum anderen befähigen sie die Pflanze, je Einheit des aufgenommenen Stickstoffs mehr Kohlehydrate zu produzieren, so daß sich dadurch die Wirksamkeit des Stickstoffs in der Pflanze erhöht.

3. Die Wirkung stickstoffhaltiger Düngemittel.

Natronsalpeter.

Eine Düngung nur mit Natronsalpeter in einer Menge von 2,8 dz je Hektar (= 46 kg Stickstoff je Hektar) steigerte während der ersten 15 Jahre durchschnittlich den Gesamtertrag um 21,4 dz/ha und den Korn-

¹⁾ Nach 1906 Parzelle 3 b.

²⁾ Nach 1906 Parzelle 5 aa.

³⁾ Nach 1906 Parzelle 2 b.

ertrag um etwa 8,8 dz/ha gegenüber der ungedüngten Parzelle. Weit höhere Leistungen erreichte man, wenn man auch Kali und Phosphorsäure zusätzlich gab.

Tabelle 7.

Die je Kilogramm Stickstoff (mit dem Düngemittel zugeführt) erzielten Ertragssteigerungen in Kilogramm je Hektar.

1877 bis 1891	Natronsalpeter allein		Natronsalpeter und Mineralsalze	
	Woburn	Rothamsted	Woburn	Rothamsted
Einfache Gaben				
Gesamtertrag	46	43	73	73
Kornertrag	19	20	29	32
Doppelte Gaben				
Gesamtertrag	—	—	51	—
Kornertrag	—	—	18	—

Die doppelte Gabe von Natronsalpeter brachte einen noch höheren Ertrag, allerdings keine Vermehrung um das Doppelte, so daß der Stickstoff je Gewichtseinheit weniger wirksam war¹⁾ (Tabelle 9).

Das Ergebnis ähnelte dem von Rothamsted: dort wurde auch bei Ertragssteigerung die Wirksamkeit des Stickstoffs durch Kali und Phosphorsäure erhöht, aber vermindert, wenn die N-Düngermenge über ein bestimmtes Maß hinausging. Trotz des Unterschiedes in der Bodenart stimmen die Bilder einigermaßen überein. Die Versuche waren in Rothamsted 25 Jahre eher begonnen worden als in Woburn, allerdings hatte man bis zum Jahre 1868 noch keine Versuche mit Natronsalpeter angesetzt. Die Steigerungen des Gesamtertrages und des Kornertrages je Kilogramm zugeführten Stickstoffs unterschieden sich bei einer Zusammenfassung von je fünf Jahren nur wenig von denjenigen, die in Rothamsted

Tabelle 8

	Natronsalpeter allein		Natronsalpeter und Mineralsalze	
	Woburn	Rothamsted	Woburn	Rothamsted
Einfache Düngung				
Gesamtertrag	18	36	38	49
Kornertrag	6	14	15	23

¹⁾ Nach den ersten fünf Jahren (d. h. nach 1883) lassen sich die doppelt starken Düngungen nicht genau mit den einfachen vergleichen, weil sie auf einer halben Parzelle zur Anwendung kamen, die im vorübergehenden Jahr keine Stickstoffgabe erhalten hatte, während einfache Düngungen stets auf einfache des Vorjahres folgten. Daher kamen bei der einfachen Düngung „Nachwirkungen“ zum Ausdruck, während dies bei den doppelten Gaben nicht der Fall war.

Tabelle 9.
Steigerung des Gesamt- und Kornertrages der Gerste durch Natronsalpeter und Stallmist.

Stückung dz ha	Andere Düngemittel	Parzellen	Gesamt- ertrag dz ha	Zunahme gegenüber ohne Stickstoff ¹⁾ dz ha	Kornertrag dz ha	Zunahme gegenüber ohne Stickstoff ¹⁾ dz ha	Steigerung des Gesamt- ertrages je Einheit des mit dem Düngemittel zugeführten Stickstoffs	Von der Pflanze aus dem Düngemittel auf- genommen	Steigerung des Kornertrages je Einheit des mit dem Düngemittel zugeführten Stickstoffs	Von dem Korn aus dem Lüngemittel auf- genommen
Die ersten 15 Jahre 1877 bis 1891										
0,46	Keine Rothamsted . . .	3	51,1	21,1	22,0	8,6	46	81 ¹⁾	19	34
0,46	Mineralsalze Rothamsted .	6	37,5	21,0	17,6	9,5	13		20	
0,92	Mineralsalze	4 AA	62,9	33,5	26,9	13,4	73	89 ¹⁾	29	36
1,23	Mineralsalze	9	56,3	35,2	25,8	15,2	73		32	
	Mineralsalze	11 b	76,2	46,8	30,2	16,7	51	82 ¹⁾	18	29
	Stallmistdüngung		50,7	21,2	23,1	9,6	17	64	8	28
Die zweiten 15 Jahre 1892 bis 1906										
0,46	Keine Rothamsted . . .	3	33,6	15,9	15,0	7,0	34	59	15	26
0,46	Mineralsalze Rothamsted .	6	33,6	18,8	15,0	8,3	39		17	
0,92	Mineralsalze	9	49,6	27,1	21,7	11,2	59	77	24	32
1,23	Mineralsalze	11 b	54,6	33,8	24,1	14,6	70		30	
	Stallmistdüngung		59,4	37,3	25,5	15,0	41	63	16	25
	Stallmistdüngung		51,3	29,2	23,3	12,8	24	82	10	36
Die letzten 20 Jahre 1907 bis 1926										
0,23	Keine	3 B	17,8	4,3	6,7	1,6	18		7	
0,46	Keine Rothamsted . . .	3 A	21,9	8,4	8,0	2,9	18	79	6	28
0,23	Mineralsalze	6	31,8	17,3	13,8	7,0	36		14	
0,46	Mineralsalze Rothamsted .	9 A	26,7	9,6	10,8	4,0	42	89	18	38
1,23	Mineralsalze	11 b	34,4	17,4	13,4	6,7	38	92	15	35
	Stallmistdüngung		49,1	23,6	22,1	11,2	49		23	
	Stallmistdüngung		41,1	24,0	17,0	10,3	19	94	8	40

¹⁾ Nur zehn Jahre, von 1882 bis 1891.²⁾ D h. Überschub über die Erträge der ungetüngelten Parzellen 1 und 7 und der Parzelle 4, die nur Mineralsalze erhalten hatte.

Die Angaben von Rothamsted beziehen sich auf die Parzellen 1AA und 10 sowie auf 4AA und 40. Die Einteilung der Jahre ist für Rothamsted so festgesetzt wie für Woburn, wenn es auch dort nicht die „ersten 15 Jahre“ waren, da der Versuch in Rothamsted schon 1852 begonnen hatte

während der ersten 25 Jahre erreicht waren. Die Ergebnisse der beiden Stationen sind für die Jahre 1877 bis 1891 in Tabelle 7 dargestellt.

Nach dieser ersten Periode begann eine Abnahme in der Wirksamkeit des Natronsalpeters, das Sinken machte sich in Woburn schneller bemerkbar als in Rothamsted. Die Zunahme je Kilogramm Stickstoff in den letzten 20 Jahren (1907 bis 1926) ist in Tabelle 8 dargestellt.

Die für die letzten 20 Jahre (1906 bis 1927) in Woburn ermittelten Werte können nicht genau mit den früheren verglichen werden, teils, weil die Düngung mit Stickstoff später erfolgte als in den Jahren vorher¹⁾, teils aber hauptsächlich deshalb, weil eine Änderung in der Düngung eingetreten war. Die Werte für 100 kg Stickstoff hängen von der Menge der gegebenen Düngemittel ab und brauchen für 22 kg nicht unbedingt dieselben wie für 44 kg Düngemittel zu sein.

Tabelle 9a.

Die durchschnittliche, jährlich von den Pflanzen aufgenommene Stickstoffmenge in Kilogramm je Hektar.

Parzelle	Gerste									Gesamtergebnis 1882 bis 1926
	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926	
1 und 7	32	29	26	19	20	12	17	14	9	898
4 a	29	32	32	25	24	14	24	19	13	1064
2 a	57	65	53	14	2	—	—	—	—	—
3 a	64	56	57	47	40	30	24	21	20	1799
5 a	60	64	67	19	7	—	—	—	—	—
6	75	67	73	62	53	29	32	28	24	2209
9 mit N.	100	86	94	84	81	44	37	36	30	2954
9 ohne N.	54	45	52	37	35	25	26	22	18	1561
11 b	78	55	72	57	58	49	45	48	31	2466

Die aus den vollständig gedüngten Parzellen erhaltenen Werte betragen je Einheit des zugeführten Stickstoffs 38 und 42 Einheiten des Gesamtertrages und 15 und 18 Einheiten des Kornertrages. Die Zahlen ändern sich je nach der Höhe der Stickstoffgabe; sie liegen ungefähr 20% unter den entsprechenden Werten in Rothamsted. Sie dürften deshalb von einigem Interesse sein, weil sie nicht nur genau so hoch sind wie diejenigen Werte, die in der ersten Periode durch starke Düngung

¹⁾ Durchschnittliche Daten der Düngung mit Natronsalpeter und schwefelsaurem Ammoniak:

	Dauernder Weizenbau	Dauernder Gerstenbau
1880 bis 1886	2. April	22. April
1887 bis 1896	1. Mai	8. Mai
1897 bis 1906	12. Mai	23. Mai
1907 bis 1916	25. Mai	30. Mai
1917 bis 1926	8. Juni	15. Juni

Tabelle 10.
Steigerung des Gesamt- und Kornertrages bei Weizen durch Natronsalpeter und Stallmist.

Stickstoff- düngung	Andere Düngemittel	Par- zelle	Gesamt- ertrag	Zunahme gegenüber ohne Stickstoff ¹⁾	Kornertrag	Zunahme gegenüber ohne Stickstoff ¹⁾	Steigerung des Gesamt- ertrages je Einheit des mit dem Düngemittel zugeführten Stickstoffs	Von der Pflanze aus dem Düngemittel aufgenommen	Steigerung des Korn- ertrages je Doppelzent- ner mit dem Düngemittel zugeführten Stickstoffs	Von dem Korn aus dem Düngemittel aufgenommen
dz/ha			dz/ha	dz/ha	dz/ha	dz/ha				
Die ersten 15 Jahre 1877 bis 1891										
0.46	Keine	3	47.3	15.4	15.4	4.8	33	—	10	8
—	Mineralsalze	6	64.8	32.1	21.7	10.9	70	28	24	15
0.92	Mineralsalze	9	78.1	45.4	23.0	12.9	49	43	14	—
1.23	Stallmistdüngung	11 b	53.8	21.0	18.5	7.8	17	—	6	12
Die zweiten 15 Jahre 1892 bis 1906										
0.46	Keine	3	35.3	16.0	12.2	5.0	35	40	11	12
—	Mineralsalze	6	44.3	26.6	16.5	10.2	58	48	22	18
0.92	Mineralsalze	9	53.0	35.3	19.1	12.9	38	—	14	—
1.23	Stallmistdüngung	11 b	46.3	28.6	16.6	10.3	23	55	8	20
Die letzten 20 Jahre 1907 bis 1926										
0.23	Keine	3 B	27.2	11.6	9.8	3.9	50	41	17	14
0.46	Keine	3 A	—	—	—	—	—	—	—	—
0.23	Mineralsalze	6	31.6	15.0	11.5	5.7	65	46	25	17
0.46	Mineralsalze	9 A	33.8	17.2	11.5	5.7	37	—	12	—
1.23	Stallmistdüngung	11 b	38.2	21.7	12.9	7.1	18	57	6	19

¹⁾ Nur 10 Jahre, von 1892 bis 1891.

²⁾ D h Überschuß über die Erträge der ungedüngten Parzelle 1 und 7 und der Parzelle 4, die nur Mineralsalze erhalten hatte.

Die Angaben von Rothamsted beziehen sich auf die Parzellen 1 A A und 10 sowie auf 4 A A und 40. Die Einteilung der Jahre ist für Rothamsted so festgesetzt wie für Woburn, wenn es auch dort nicht die „ersten 15 Jahre“ waren, da der Versuch in Rothamsted schon 1852 begonnen hatte.

Tabelle 10a.

Die durchschnittliche, jährlich von den Pflanzen aufgenommene Stickstoffmenge in Kilogramm je Hektar.

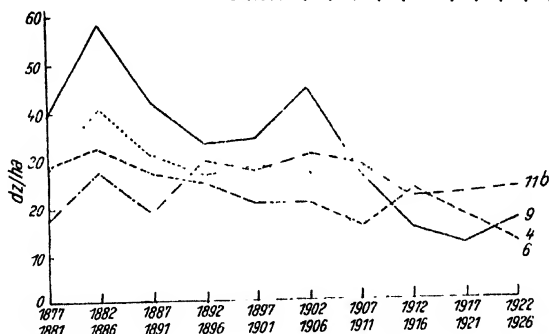
Parzelle	Weizen									Gesamt- ergebnis- 1882 bis 1926
	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926	
1 und 7	37	30	22	21	20	21	19	19	8	989
4 a	39	30	20	19	18	19	16	21	10	963
3 a	60	52	40	45	36	37	27	31	18	1725
6	80	68	54	60	50	38	31	40	20	2218
11 b	69	66	49	54	54	46	37	49	20	2222

von 92 kg je Hektar erreicht wurden, sondern weil sie auch ziemlich genau mit den Durchschnittsergebnissen unserer in jüngster Zeit in praktischen Betrieben durchgeführten Versuche und den kürzlich in Deutschland errechneten Mittelwerten übereinstimmen.

Tabelle 11.

Steigerung des Kornertrages je Einheit Stickstoff.

Woburn: Dauer-Gerstenversuch in den letzten 20 Jahren	15—18
Rothamsted: Dauer-Gerstenversuch in den letzten 20 Jahren (Parzelle 4 AA) . .	23
Letzte Feldversuche in England bei einer Gabe von 1,25 dz schwefelsauren Ammoniak je Hektar	15
Die letzten Versuche in Deutschland. Lemmermann	18,5
Nolte	17



- — — — — Tatsächlicher Ertrag, Parzelle 4.
 Steigerung des Ertrages, Parzelle 6 über Parzelle 4.
 - - - - - Steigerung des Ertrages, Parzelle 9 über Parzelle 4.
 - . - . - Steigerung des Ertrages, Parzelle 11 b über Parzelle 4.

Abb. 2a.

Steigerung des Gesamtertrages bei Gerste
durch Natronsalpeter- und Stallmist-Düngung.

Diese Zahlen stellen natürlich nur Durchschnittswerte dar; die wirklichen Werte weichen von Jahr zu Jahr beträchtlich ab. Wenn die Parzellen ohne Stickstoff hohe Erträge liefern, sind die Unterschiede größer als wenn sie schlechte Ernten bringen. Die tatsächlichen Ergebnisse sind durch Abbildung 2a und 2b dargestellt. Man hätte annehmen können, daß die verminderte Düngung nach 1907 gegenüber der ursprünglichen

Düngung einen entsprechend größeren Unterschied der Erträge hervorgerufen würde, aber diese Vermutung bestätigte sich nicht. Die Beobachtung, daß immer in den Jahren mit geringen Ernten die Erträge langsamer abnehmen als in den Jahren mit höheren Ernten zeigt, daß stickstoffhaltige Düngemittel die Ernten auf derselben Höhe halten und die durch den einseitigen Anbau bedingte Verschlechterung in einem gewissen Ausmaß abschwächen.

Diese bei Stickstoffgaben erzielten Ergebnisse stehen im Gegensatz zu denen bei Mineralsalzdüngung; hier hat der Ertrag die Tendenz zum Steigen, während er ohne Mineralsalze fällt.

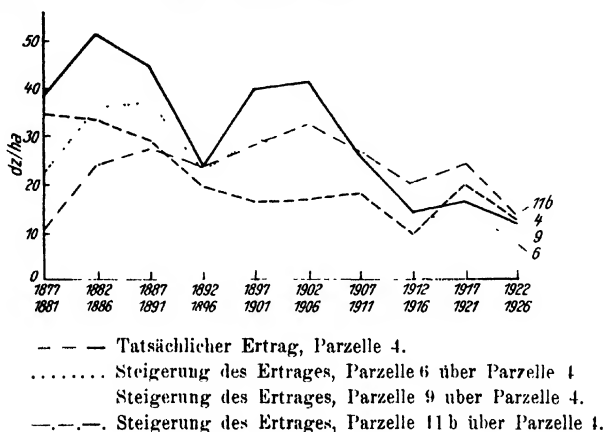


Abb. 2b

Steigerung des Gesamtertrages bei Weizen
 durch Natronsalpeter- und Stallmist-Düngung.

Ertragssteigerungen je Kilogramm aufgenommenen Stickstoffs.

Angaben über die von den Pflanzen aufgenommene Stickstoffmenge finden wir später (S. 184).

Die Ertragssteigerungen je Kilogramm aufgenommenen Stickstoff sind in Tabelle 10 gegeben. Diese zeigen viel weniger Abweichungen als die vorher aufgeführten Erträge. Es ist hieraus ersichtlich, daß der verminderte Einfluß der zweiten Stickstoffgabe und das Nachlassen der Wirkung in der zweiten und dritte Periode vom Jahre 1892 an nicht einem Versagen des Stickstoffes in seiner Wirkung innerhalb der Pflanze zuzuschreiben ist, sondern veranlaßt wird durch mangelnde Fähigkeit der Pflanze, den Stickstoff aufzunehmen.

Schwefelsaures Ammoniak.

Während der ersten 15 Jahre läßt sich die Hauptwirkung des schwefelsauren Ammoniaks mit der des Natronsalpeters vergleichen. Die Wirkung seines Stickstoffs beträgt, wenn man diejenige des Natronsalpeters = 100 setzt, wie folgt:

Für 46 kg Stickstoff	allein	102
	+ Mineralsalze . .	87
Für 92 kg Stickstoff	+ Mineralsalze . .	96

Beinahe sofort hinterher setzte eine Verminderung der Erträge ein. Dieses rasche Sinken ist in Tabelle 12 dargestellt. Bis zum Jahre 1891 hatte das schwefelsaure Ammoniak auf Parzelle 2 eine etwas größere Wirkung als Natronsalpeter auf Parzelle 3. Vom Jahre 1892 an ließ die Wirkung nach, und innerhalb weniger Jahre sanken die Ernten so weit herunter, daß die Erträge schließlich fast ganz schwanden; sogar mit dem Unkraut war dies der Fall, nur Ackerspörgel (*Spergula arvensis*) machte hierbei eine Ausnahme. In dem gesamten Bereich der britischen Feldversuche ist nichts so auffallend gewesen wie diese Gerstenparzellen, von denen die Parzellen 1, 2 und 3 einander am nächsten standen.

Tabelle 12.
Kornerträge in Doppelzentnern je Hektar.
Ein Vergleich zwischen schwefelsaurem Ammoniak und Natronsalpeter.

Parzelle		1889	1890	1891	1892	1893	1894	1895	1896	1897
	Keine Mineralsalze									
1	Ungedüngt	6,4	17,5	11,8	10,6	8,3	10,6	4,6	5,8	3,4
3	Natronsalpeter allein . . .	16,0	22,7	16,1	24,5	11,7	21,2	10,4	11,6	12,6
2	Schwefelsaures Ammoniak allein	16,9	23,8	18,3	20,3	8,4	16,4	8,3	6,5	5,5
	Mineralsalze									
4	Ohne Stickstoff	7,7	19,5	15,7	18,2	9,9	13,7	8,7	7,9	6,2
6	Natronsalpeter	20,1	31,4	24,6	32,5	13,8	29,3	18,1	18,0	20,0
5	Schwefelsaures Ammoniak .	19,9	30,2	22,9	29,4	11,9	29,2	9,4	9,4	7,4

Beim Betrachten der Parzellen hat man zunächst den Eindruck, daß die Gerste auf der sauren Parzelle 2 nicht gekommen sei. Das ist aber nicht der Fall. Die Saat hat gekeimt und die Keimlinge sind vorschriftsmäßig gekommen. Das Wurzelsystem ist sehr verkümmert; die Wurzeln sind sehr dick und tragen nur wenig Wurzelhaare. Die Blätter bekommen an den Spitzen eine rötliche und an der Basis der Blattscheide eine gelbe Färbung; bald können sie nicht mehr in dem dicht gewachsenen Ackerspörgel (*Spergula*) erkannt werden, der die ganze Parzelle überwuchert und eine Höhe von 15 cm erreicht. Von April an ist die Gerste nicht zu sehen. Beim sorgfältigen Suchen in dem Unkraut merkt man aber, daß die Gerste noch am Leben ist und ihre normalen Wachstumsstadien durchmacht. Obgleich die Pflanze nur 20 bis 25 cm hoch ist, wird sie reif und bringt dünne Ähren mit winzigem Korn hervor, ungefähr zur gleichen Zeit wie die normalen Pflanzen auf den angrenzenden Parzellen. Diese verkümmerten Pflanzen sind sehr merkwürdig; sie werden jetzt genau untersucht und sind sicher die kleinsten Gerstenpflanzen, die jemals gewachsen sind.

Die drei Eigenschaften: fleckweises Auftreten, verkrüppeltes Wurzelsystem mit einem auffallenden Mangel an Wurzelhaaren und verkümmertes Wachstum sind jetzt als Merkmale für Bodensäure festgestellt worden, so daß man sofort die Felder erkennen kann, die eine Kalkung nötig haben.

Ein Vergleich der Parzellen 5 und 6 (Schwefelsaures Ammoniak oder Natronsalpeter mit Mineralsalzen) zeigt, daß das Sinken der Erträge bei schwefelsaurem Ammoniak sich im Verhältnis zu Natronsalpeter genau so bald bemerkbar machte wie bei den Parzellen 2 und 3. Zusätzliche Mineralsalzgaben vermochten also den schädlichen Einfluß der Säure weder zu hindern noch aufzuschieben. Im Gegenteil, die Tendenz ging sogar in entgegengesetzter Richtung. Die Mineralsalze verhinderten es immerhin, daß die absoluten Erträge auf den sauren Parzellen zu stark absanken, und bis zum Ende der Versuchsperiode konnte immer noch ein gewisser Ertrag geerntet werden, wenn er auch in einigen Jahren nur eine Höhe von 1,34 oder 2,01 dz je Hektar erreicht hatte. Die Ernten sind nicht im einzelnen untersucht worden; aber weder der Prozentsatz des Stickstoffs im Korn noch das Verhältnis von Kornertrag zum Gesamtertrag weichen bei diesen Parzellen mit saurem Boden merklich von den bei anderen Parzellen gewonnenen Ergebnissen ab (Tabelle 21).

4. Die Wirkung von Kalk auf Parzellen mit saurem Boden.

1897 hatte sich die Einwirkung der Säure auf die Parzellen mit schwefelsaurem Ammoniak deutlich bemerkbar gemacht; die Parzellen wurden deshalb nochmals geteilt und einzelne von diesen Stücken wurden mit Kalk behandelt.

Die einfachste Wirkung erzielte man mit einer Gabe von 25 dz Kalk je Hektar auf eine Parzelle, die außerdem noch volle Minereraldüngung erhielt (Parzelle 5 AA). Für einen Zeitabschnitt von acht bis zehn Jahren nach der 1905 zum ersten Mal angewendeten Kalkung¹⁾ wurde durch diese der Ertrag bis zu der Höhe der entsprechenden Natronsalpeter-Parzelle (Parzelle 6) gesteigert; aber dann machten sich Anzeichen der Abnahme bemerkbar. Deshalb wurde 1916 eine zweite Kalkdüngung ebenfalls in einer Stärke von 25 dz je Hektar durchgeführt. Bis zum Jahre 1926, in dem der Versuch zum Abschluß gelangt war, hielt diese zweite Düngung den Ertrag auf der von der Natronsalpeter-Parzelle erreichten Höhe.

Bei Kalkdüngungen mit 50 dz je Hektar wurden die Wirkungen verwickelter (Abb. 3). Die ersten Düngungen von 1897 brachten Ergebnisse, wie sie eben beschrieben worden sind; aber die zweiten Düngungen vom Jahre 1912 zeigten erhebliche Abweichungen. Mit und ohne Mineralsalzgaben steigerten sie beide den Ertrag weit über den der entsprechenden Parzelle mit Natronsalpeter (Parzelle 6B); für zwei oder drei Jahre behielten sie diesen Vorrang; dann sanken sie ungefähr bis zu der Höhe der mit Natronsalpeter gedüngten Parzelle und verblieben dort bis zum Schluß.

Angenommen, die anfängliche Wirkung wäre tatsächlich vorhanden, so muß man dem Kalk eine Zersetzung der Bodensubstanz zuschreiben: Entweder die wohlbekannte schnelle Zersetzung der organischen Stoffe, die sich in der Bildung leichter zugänglicher Stickstoffverbindungen auswirkt oder in der Abgabe verfügbaren Kalis von schwer zersetzlichen

¹⁾ Die Düngung erfolgte im Mai und hatte im ersten Jahre noch keinen Einfluß.

Bodenkomplexen — eine Wirkung, die gewöhnlich oft in den Lehrbüchern beschrieben wird, für die in England aber immer wenig wirkliches Beweismaterial erbracht worden ist. Die Tatsache, daß ein gesteigerter Ertrag durch Mineralsalzgaben erzielt wird, deutet darauf hin, daß die Wirkung eigentlich darin besteht, mehr Stickstoff als Kali freizumachen. Im Vergleich zu der neutralisierenden Wirkung des Kalkes sind diese Einflüsse jedoch alle von untergeordneter Bedeutung.

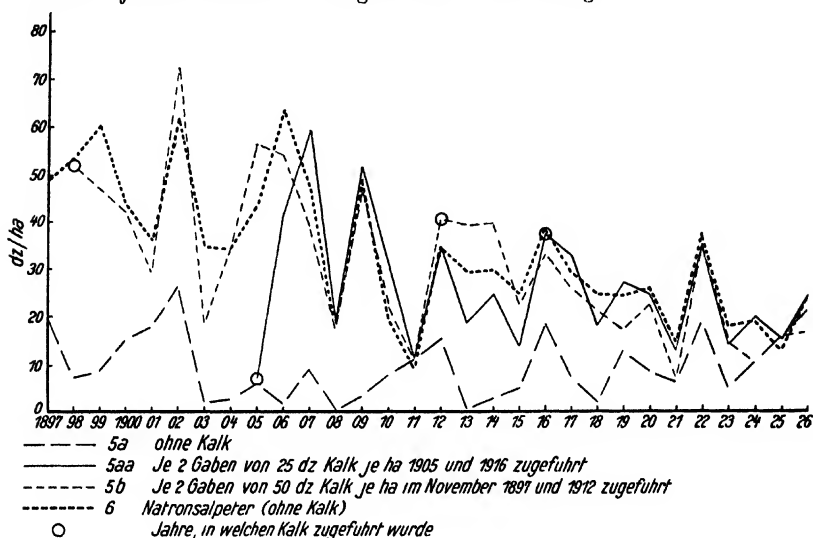


Abb. 3.

Wirkungen von Kalk in großen und kleinen Gaben auf dem sauren Boden in Woburn mit schwefelsaurem Ammoniak und mineralischer Düngung.

Es ist bemerkenswert, was für einen großen Erfolg schwache Kalkdüngungen gezeitigt haben. Sie haben Erträge erzielt, die weit höher waren als diejenigen, die mit einer Düngung von schwefelsaurem Ammoniak allein erreicht worden waren. Die von 1909 bis 1912 durchgeführten drei Düngungen hielten die Ernten bis 1920 auf der gleichen Höhe, d. h. die 18 dz schienen über zehn Jahre wirksam zu sein, während die Düngung mit 12 dz vom Jahre 1923 den Ertrag der Parzelle 2 BB (100 dz Kalk zusammen) übertraf, nachdem der Ertrag vorher auf den der Parzelle 2 A gesunken war.

Diese schwachen Düngungen wurden weit sparsamer ausgenutzt als die starken; allerdings würden sie für die Verwendung in der Praxis wahrscheinlich weniger vorteilhaft sein, da sie geringere Erträge brachten. Infolge der jährlichen Schwankungen im Ertrage ist es nicht möglich, die Wirksamkeitsdauer des Kalkes mit Sicherheit festzustellen.

Die Erträge der mit Kalk behandelten Parzellen fielen ab gegenüber den mit Natronsalpeter gedüngten Parzellen; aber selbst nach 15 Jahren sanken sie nicht bis zu den geringen Erträgen der Parzellen herunter, die nicht mit Kalk gedüngt waren.

Nur auf den mit schwefelsaurem Ammoniak behandelten Parzellen zeigten sich diese deutlichen Wirkungen des Kalkes. Die Parzellen, die Mineralsalze, aber keinen Stickstoff, erhalten hatten, reagierten sehr wenig auf die Kalkdüngung; in den ersten Jahren bewirkte Kalk eine deutliche Ertragssteigerung, die auf eine Abgabe verfügbaren Stickstoffs hindeutet. Später aber sind diese Unterschiede weit geringer und mögen tatsächlich durch Verschiedenartigkeit des Bodens veranlaßt gewesen sein. Die Kalkdüngung auf der Hälfte der Parzelle 8 (ein Jahr um das andere eine doppelte Gabe schwefelsaures Ammoniak und nur im Jahre 1897 und außerdem im Jahre 1912 eine Kalkdüngung von 50 dz je Hektar) steigerte in den Jahren mit zusätzlicher Düngung von schwefelsaurem Ammoniak zwar die Erträge, allerdings nicht bis zu der Höhe der entsprechenden Parzelle mit einer Düngung von Natronsalpeter (Parzelle 9). In den Jahren, in welchen Stickstoff nicht zur Wirkung kam, waren die Erträge des gekalkten Stückes ebenso gut wie diejenigen der ohne Stickstoff behandelten Parzelle 9; anscheinend war hier kein Säurerückstand zu beobachten.

5. Stallmistdüngung.

Die ursprüngliche Versuchsanordnung sah zwei Parzellen mit Stallmistdüngung vor, die ungefähr 4 bzw. 8 Tonnen (1 Tonne = 907,1853 kg) je acre (= 40,4678 a) mit 55 und 110 Pfd. Stickstoff (= 62 bzw. 124 kg N je Hektar) erhielten. Der Arbeitsplan wurde 1882 insofern erweitert, als noch Versuche mit Nachwirkungen angesetzt wurden. Die vier Parzellen blieben bis 1887 in Bearbeitung, dann wurden die beiden schwach gedüngten Parzellen aus dem Versuch herausgenommen.

Tabelle 13 bringt die in den zehn Versuchsjahren beobachtete Steigerung des Gesamtertrages in Doppelzentnern je Hektar¹⁾.

Tabelle 13

	Ertragssteigerung gegenüber den ungedüngten Parzellen (1 und 7)			Ertragssteigerung gegenüber der nur mit Mineral- salzen gedüngten Parzelle (4)		
	Einfache Gaben 62 kg ha Stickstoff	Doppelte Gaben 124 kg ha Stickstoff	Verhältnis	Einfache Gaben 62 kg/ha Stickstoff	Doppelte Gaben 124 kg/ha Stickstoff	Verhältnis
Die ersten 5 Jahre 1877 bis 1881. . . .	5,5	15,4	1 : 2,8	7,9	17,8	1 : 2,2
Die zweiten 5 Jahre 1882 bis 1886. . . .	9,5	24,5	1 : 2,6	11,8	26,9	1 : 2,4

¹⁾ Die Stallmistdüngung wurde nicht immer in derselben Weise durchgeführt. In der Zeit von 1877 bis 1888 wurde Stallmist im Herbst vor der Aussaat untergepflügt, während der darauffolgenden Periode von 1889 bis 1906 wurde Stallmist als Kopfdüngung im Frühjahr verwendet. Ein eindeutiger Vergleich läßt sich nicht durchführen, aber die durchschnittlichen Erträge sind in beiden Zeiträumen fast dieselben, so daß zwischen den beiden zur Anwendung gekommenen Düngungsmethoden irgendwelcher Unterschied prak-

Das obige Resultat weicht insofern von dem bei einer Düngung mit Natronsalpeter gewonnenen Ergebnis ab, als sich hier die zweite Gabe gegenüber der ersten genau so, wenn nicht noch stärker, in ihrer Wirkung zeigt und damit wird auch der von der Praxis vertretene Standpunkt, dem Boden lieber große Stallmistgaben aber kleine Mengen künstlicher Düngemittel zuzuführen, gerechtfertigt. Nach Ablauf der Versuchsjahre fiel ein zweiter Unterschied deutlich in die Augen: Stallmist behielt seine Wirksamkeit bei, hier zeigte sich nicht ein solches Absinken wie bei einer Düngung mit Natronsalpeter und schwefelsaurem Ammoniak. Vom Beginn bis fast zum Abschluß des Versuches hindurch ergab ein Teil Stickstoff in der Stallmistdüngung regelmäßig etwa 20 bis 24 Anteile des Gesamtertrages und 10 Anteile des Kornertrages. Natronsalpeter hingegen büßte im Laufe der Zeit an Wirksamkeit ein und ergab in den letzten Jahren nur 35 Teile des Gesamtertrages und 11 bis 15 Teile des Kornertrages gegenüber 73 Anteilen des Gesamtertrages und 29 Anteilen des Kornertrages in den früheren Jahren.

Wenn man salpetersaures Natron = 100 einsetzte, ließ sich doch durch die eben erwähnten zwei Unterschiede in der Wirkung kein festumgrenztes Äquivalent für den Stickstoff in der Stallmistdüngung festlegen. Die erhaltenen Werte stiegen von 23 in den ersten 15 Versuchsjahren (Parzelle 6 mit einer Stickstoffmenge von 46 kg je Hektar wurde als Grundlage für den Vergleich benutzt) bis auf 70 in den letzten 15 Jahren, während die Wirkung des Natronsalpeters erheblich nachgelassen hatte. Der frühere niedrige Wert hat praktisch eine größere Bedeutung als die späteren höheren; er wurde 1932 durch die Kohlversuche, bei welchen schwefelsaures Ammoniak als Vergleich herangezogen wurde, bestätigt. Die Zahlen sind folgende:

Gerste, die ersten 15 Jahre . 23 (Natronsalpeter)

Kohl, ein Jahr 22,4 (schwefelsaures Ammoniak)

Die Zahl gibt die Wirkung nur für das Jahr an, in dem die Düngung erfolgte; bei Berücksichtigung von Nachwirkungen würde sie sich erhöhen.

Im Vergleich zu den künstlichen Düngemitteln zeigt die Stallmistdüngung noch eine besondere Eigentümlichkeit in ihrer Fähigkeit, den Kohlenstoff- und Stickstoffgehalt des Bodens für eine lange Anbauperiode

tisch nicht besteht. Die Zahlen der unten aufgeführten Erträge beziehen sich auf Doppeltner je Hektar.

	Untergepflügte Düngung 1877 bis 1888	Kopfdüngung 1889 bis 1906
Weizen: Gesamtertrag . .	52,3	48,6
Kornertrag . .	17,8	17,4
Gerste: Gesamtertrag . .	50,5	51,3
Kornertrag . .	23,0	23,4

Die Stallmistdüngung wurde von 1906 an bei Gerste untergepflügt, bei Weizen hingegen kam sie als Kopfdüngung zur Anwendung.

hindurch auf der gleichen Höhe zu halten. Bei Parzelle 11 b zeigte sich weder eine Ab- noch eine Zunahme von Kohlenstoff und Stickstoff, bei allen anderen Parzellen dagegen ein Verlust an beiden.

Leider wurde die Parzelle 10 aus dem Versuch herausgenommen, so daß wir nicht beurteilen können, ob eine geringere Stallmistdüngung genügt haben würde, um den Kohlenstoff- und Stickstoffgehalt des Bodens in der gleichen Weise zu erhalten (S. 248).

6. Rapskuchen als Düngung.

1888 wurde mit der Stallmistdüngung (62 kg Stickstoff je Hektar) auf Parzelle 10 b abgebrochen und an ihrer Stelle Rapskuchendüngung gegeben. In den darauffolgenden 17 Jahren wurde mit einer Gabe von 92 kg Stickstoff je Hektar gedüngt, vom Jahre 1906 an nur mit dem vierten Teil dieser Menge.

Der Erfolg der stärkeren Düngung zeigte sich darin, daß die Ernte annähernd bis zu der Ertragshöhe der Parzelle 11 b, die Stallmist in einer Menge von 123 kg je Hektar erhalten hatte, gesteigert wurde, ein Beweis dafür, daß der Stickstoff im Rapskuchen genau die gleiche Wirkung besitzt wie im Stallmist (Tab. 14). Hier kann allerdings die Einwendung gemacht werden, daß die vor 1888 gegebenen Düngemittel sicherlich Rückstände im Boden übrig gelassen haben, deren Wirkung sich nicht bestimmen läßt.

Nach 1911, d. h. nach dem 22. Jahre der ununterbrochenen Rapskuchendüngung, setzte eine bemerkenswerte Veränderung ein: Die Erträge begannen rasch zu sinken und kamen bald auf die Ergebnisse der Parzelle 4 (Mineralsalze ohne Stickstoff), ein Zeichen dafür, daß der Stickstoff seine Wirkung verloren hatte. Wir können uns diesen Ausfall nicht erklären. Die Parzelle wurde saurer und dieses mag die unmittelbare

Tabelle 14.

Die Steigerung der Erträge je Kilogramm Stickstoff der Stallmist- und Rapskuchenparzelle, verglichen mit Parzelle 4, die nur Mineraldüngung erhielt.

	1890 bis 1899	1900 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
Gesamtertrag						
Rapskuchen						
92 kg/ha Stickstoff	24	27	—	—	— 4	—
23 kg/ha Stickstoff	—	—	41	13	—	— 1
Stallmist						
123 kg/ha Stickstoff	24	22	—	—	—	—
92 kg/ha Stickstoff	—	—	31	24	24	24
Kornertrag						
Rapskuchen						
92 kg/ha Stickstoff	10	12	—	—	—	—
23 kg/ha Stickstoff	—	—	22	4	— 3	— 1
Stallmist						
123 kg/ha Stickstoff	10	10	—	—	—	—
92 kg/ha Stickstoff	—	—	15	9	10	10

Ursache gewesen sein. Aber wir können bis heute nicht sagen, warum eine Säurezunahme stattfand und ob eine Unwirksamkeit des Stickstoffs in der organischen Substanz dann einsetzt, wenn die Düngergaben bis unter eine bestimmte Grenze vermindert werden.

Die erzielten Ertragssteigerungen, berechnet je Kilogramm des mit dem Düngemittel gegebenen Stickstoffs, zeigt Tabelle 14.

7. Die den Pflanzen aus dem Boden und aus den Düngemitteln zugeführte Stickstoffmenge.

Die Stickstoffaufnahme der Pflanze ist im Laufe der Arbeit nicht untersucht worden, aber es ist sehr günstig, daß Proben von Gerstenkörnern der wichtigeren Parzellen seit 1882 aufgehoben worden sind; in einigen Fällen sind sogar seit diesem Zeitpunkt fast vollständige Serien vorhanden. Diese sind auf Stickstoffgehalt untersucht worden (Tabelle 44). Strohproben wurden nicht aufgehoben und Stickstoffbestimmungen sind im Laufe des Versuchs nicht gemacht worden; man weiß, daß der Hauptteil des Stickstoffs vom Stroh nach dem Korn wandert. Eine Anzahl der in Rothamsted durchgeführten Analysen hat den Beweis erbracht, daß sich annähernd 70% des Gesamtstickstoffs gewöhnlich im Korn und 30% im Stroh befindet. Wenn wir diese Zahl annehmen und addieren den für Stroh errechneten Wert und den des Korns, so erhalten wir mit den Ergebnissen von Tabelle 15 die von den Pflanzen in den verschiedenen Jahren aufgenommene Stickstoffmenge. Die Art der Ermittlung dieser Werte schließt naturgemäß jede Möglichkeit aus, die Stickstoffaufnahme in den einzelnen Jahren genau zu erfassen. Wir können daher auch nicht sagen, wie sie durch die Witterungsverhältnisse beeinflußt wird. Der Fehler wird allerdings geringer, wenn man einen Zeitraum von fünf oder mehr Jahren überblickt und nur die Unterschiede zwischen verschiedenen Parzellen berücksichtigt.

Parzellen ohne zusätzliche Stickstoffgaben.

In der Annahme, daß das Korn 70% der gesamten aufgenommenen Stickstoffmenge enthält, haben die auf den ungedüngten und den nur mit Kali und Phosphat gedüngten Parzellen gewachsenen Pflanzen jedes Jahr aus dem Boden die unten angegebenen, auf Kilogramm je Hektar berechneten Stickstoffmengen aufgenommen.

Die Stickstoffmenge in Kilogramm je Hektar und Jahr, in der Gerstenernte (Korn und Stroh) dem Boden entzogen.* (45 Jahre, von 1882 bis 1926.)

	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926	Summe
Ungedüngte Parzelle . .	31	29	27	21	20	11	14	13	8	870
Parzelle nur mit Mineraldüngung	29	32	33	25	23	14	24	19	13	1060

Die Stickstoffaufnahme fiel von einer Periode zur nächsten ab. Eine Ausnahme bildeten die Jahre 1907 bis 1911; für diesen Zeitraum war in den meisten Jahren Goldthorpe angebaut worden, die die Erträge außerordentlich drückte. Das Abfallen geschieht nicht gleichmäßig; zwischen einigen Zeiträumen zeigt sich eine stärkere Abnahme als zwischen anderen. Am auffallendsten ist vielleicht die große Stickstoffmenge aus der ungedüngten Parzelle.

Nehmen wir an, die Stickstoffaufnahme der ersten fünf Jahre (1877 bis 1881) wäre dieselbe wie für den Zeitraum von 1882 bis 1896, so konnte man in 50 Jahren bei den Pflanzen auf den beiden nachstehend angegebenen Parzellen folgende Stickstoffaufnahme feststellen:

	Kilogramm je Hektar
Ungedüngte Parzelle	1060
Nur mit Mineralsalzen gedüngte Parzelle	1230

Tabelle 15.

Die von 1883 bis 1926 in Zeiträumen von fünf Jahren festgestellte, nach Kilogramm je Hektar berechnete, durchschnittliche Stickstoffaufnahme der Gerste, Vorausgesetzt, daß sich 70% des Stickstoffs im Korn und 30% im Stroh befinden, ergeben sich für den gesamten Stickstoffgehalt in Korn und Stroh folgende Werte:

Düngung und Parzelle	Zeitraum									Gesamter- gebnis in 50 Jahren 1877 bis 1926 ¹⁾
	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926	
Ungedüngt, Parzelle 1 und 7	32,8	29,3	25,4	19,5	20,1	12,8	16,4	14,9	8,6	1063,0
Nur Mineralsalze, Parzelle 4 a	29,1	32,5	32,8	21,7	23,3	14,1	21,0	19,0	13,3	1209,5
Nur Natronsalpeter, Parzelle 3 a	63,4	56,2	57,6	47,4	40,2	30,1	23,5	21,7	19,8	2116,5
Mineralsalze—Natron- salpeter, Parzelle 6	74,8	67,1	73,0	61,3	52,4	29,4	32,6	27,9	23,4	2583,5
Nur schwefelsaures Ammo- niak, Parzelle 2 a	57,6	64,9	52,6	14,2	1,8	—	—	—	—	—
Mineralsalze—schwefelsau- res Ammoniak, Parzelle 5 a	60,6	63,6	66,6	19,4	6,8	—	—	—	—	—
Stallmist, Parzelle 11 b	78,6	54,5	71,8	57,5	58,6	49,0	44,4	47,7	31,4	2860,5
Parzelle 9 mit Stickstoff	99,8	86,0	94,6	84,4	80,4	43,6	37,1	35,4	30,3	3462,0
Parzelle 9 ohne Stickstoff	54,1	45,2	51,9	36,9	34,3	24,2	25,3	22,4	18,3	1833,5
Stallmist 1877 bis 1887, Rapskuchen 1889 bis 1926, Parzelle 10 b	40,7	56,8	66,9	57,5	62,3	28,8	28,8	18,9	9,1	2082,5

¹⁾ Angenommen, die Werte für 1877 bis 1881 sind die gleichen wie für 1882 bis 1886.

Die Mineralsalze regten wahrscheinlich das Wachstum der Wurzeln an und veranlaßten dadurch die Pflanzen zu einer stärkeren Stickstoffaufnahme aus dem Boden.

Der Stickstoffgehalt der Pflanze stellt aber nicht nur die aus dem Boden aufgenommene Stickstoffmenge dar; eine Stickstoffmenge von etwa 5,6 kg jährlich, also 280 kg zusammen, ist in dem Samen enthalten bzw. wird mit dem Regenwasser zugeführt, so daß die Stickstoffzufuhr vom Boden aus

	Kilogramm je Hektar
Auf der ungedüngten Parzelle	784
Auf der nur mit Mineralsalzen gedüngten Parzelle	952

beträgt.

Ziehen wir diese 5,6 kg je Hektar ab, so betrug zu Versuchsbeginn die Stickstoffabgabe des Bodens an die Pflanze 22 bzw. 28 kg je Hektar, beim Versuchsabschluß dagegen nur 2 kg je Hektar auf der gedüngten und 8 kg je Hektar auf der nur mit Mineralsalzen gedüngten Parzelle. Es verblieben noch folgende Mengen im Boden:

	Prozent	Kilogramm je Hektar
Ungedüngte Parzelle	0,094	3,160
Nur mit Mineralsalzen gedüngte Parzelle	0,089	2,990

eine Menge, die bei dieser Höhe der Stickstoffabgabe für mehrere hundert Jahre genügen würde. In Wirklichkeit ist der Verlust größer als dieser, der ohne Berücksichtigung der Drainage festgestellt wurde. Trotzdem bleibt ein großer Stickstoffvorrat übrig, ganz abgesehen davon, daß auch noch die Möglichkeit besteht, daß der Stickstoff der Luft bakteriell ge-

Tabelle 16.

Stickstoffgehalt der Böden bei Gerstenparzellen 1 und 4 zu verschiedenen Zeiten.

Jahr	Ungedüngt. Parzelle 1 ¹⁾				Nur Mineralsalze. Parzelle 4			
	%	kg/ha	Tatsächlicher Verlust	Jährliche Stickstoff- Abnahme	%	kg/ha	Tatsächlicher Verlust	Jährliche Stickstoff- Abnahme
			kg/ha	kg/ha			kg/ha	kg/ha
1876	0,155	52,1	3,7	0,31	0,155	52,1	11,1	0,9
1888	0,144	48,4			0,122	41,0		
1898	0,135	45,4	13,8	0,47	0,111	37,3	7,4	0,3
1927	0,094	31,6			0,089	29,9		
1932	0,095	31,9	—	—	0,086	28,9	1,0	0,2

¹⁾ 1898 waren Proben von Parzelle 7 nicht vorhanden, deshalb ist hier nur Parzelle 1 berücksichtigt.

bunden wird. In Tabelle 16 sind für die einzelnen Zeiten der verschiedene Stickstoffgehalt des Bodens und die Höhe des Stickstoffverlustes gegeben.

Da sich bei der Probeentnahme Schwierigkeiten ergaben, kann man den für den Stickstoffverlust errechneten Zahlen keinen allzu großen Wert beilegen. Wenn die Angaben zuverlässig wären, könnte man aus ihnen Folgendes ersehen: Innerhalb der ersten 22 Jahre haben die Pflanzen auf der ungedüngten Parzelle 1 80 % des Stickstoffverlustes wieder ersetzt, während die Pflanzen auf der mit Mineralsalzen gedüngten Parzelle in den zehn Jahren von 1888 bis 1898 70 % und in dem darauffolgenden Zeitraum von 29 Jahren 50 % des Stickstoffverlustes wieder aufgenommen haben. Bei jeder Parzelle gibt es aber Perioden mit viel größeren Stick-

Tabelle 17.
50jährige Beobachtungen (1877 bis 1926)
über die Veränderungen im Stickstoffgehalt des Bodens
und über die Stickstoffmenge, die durch die Sorte aufgenommen ist.

	Keine Stickstoff-Gaben		
	Ungedüngt	Nur Mineralsalze	
	Parzelle 1 und 7	Parzelle 4	
Stickstoffgehalt des Bodens in Prozenten, 1876	0,155	0,155	
Stickstoffgehalt des Bodens in Prozenten, 1927	0,094	0,090	
Stickstoffverlust in Prozenten	0,061	0,065	
Stickstoffabgabe in Doppelzentner je Hektar .	20,5	21,8	
In den Pflanzen festgestellter Stickstoff ¹⁾ . .	7,8	9,5	
Tatsächlicher Verlust	12,7	12,3	
Von den Pflanzen aufgenommener Stickstoff in Prozenten	40,0	44,0	
Verlust in Prozenten	60,0	56,0	
	Stickstoff-Gaben		
	Künstliche Düngemittel		
	Natron- salpeter allein	Natron- salpeter und Mineralsalze	Stallmist- düngung
	Parzelle 3	Parzelle 6	Parzelle 11 b
Stickstoffgehalt des Bodens in Prozenten, 1876	0 155	0,155	0,155
Stickstoffgehalt des Bodens in Prozenten, 1927	0,105	0,109	0,151
Stickstoffabgabe in Prozenten	0,050	0,046	0,004
Stickstoffabgabe in Doppelzentner je Hektar .	16,8	15,5	1,3
Mit dem Düngemittel zugeführter Stickstoff .	23,0	18,4	53,8
Summe	39,8	33,9	55,1
In den Pflanzen festgestellter Stickstoff . . .	17,9	23,0	25,8
Verlorener oder im Boden verbliebener Stickstoff ²⁾	21,9	10,9	29,3
Die gesamte in den Pflanzen festgestellte Stickstoffmenge in Prozenten	45,0	66,0	47,0
Verbleib im Boden oder Verlust in Prozenten	55,0	34,0	53,0

¹⁾ Nach Abzug von 0,04 dz im Jahr für Saatgut und Niederschläge.

²⁾ Tatsächlicher Verlust.

stoffverlusten und geringeren Stickstoffaufnahmen. Auf die ganze Versuchszeit berechnet, haben die Pflanzen

auf der ungedüngten Parzelle . . 40% des Stickstoffverlustes
und auf der nur mit Mineralsalzen

gedüngten Parzelle 44% des Stickstoffverlustes
durch Stickstoffaufnahme aus dem Boden wieder ersetzt.

Hinsichtlich der verschiedenen Düngungsarten gibt Tabelle 17 wahrscheinlich die genauesten Schätzungen der Stickstoffaufnahme. Sie zeigen, daß auf den mit Stallmist, nur mit Natronsalpeter, nur mit Mineralsalzen gedüngten sowie auch auf den ungedüngten Parzellen ungefähr die Hälfte der Stickstoffmengen, die vom Boden abgegeben und im verabreichten Düngemittel zugeführt werden, wieder in der Ernte in Erscheinung tritt. Durch Volldüngungen wird die Stickstoffaufnahme auf ungefähr $\frac{2}{3}$ des Stickstoffverlustes erhöht.

Für diese Tabelle wird nur vorausgesetzt, daß die Proben den Stickstoffgehalt der vorliegenden Parzelle darstellen und daß sich bei den Pflanzen 70% des Stickstoffs im Korn befindet. Die Wiedergewinnung des Stickstoffs ergibt sich aus der Verbindung Boden + Düngung und nicht nur aus den verabreichten Düngemitteln. Für diese allein kann man den Stickstoffgewinn nur dann berechnen, wenn man weiterhin annimmt, daß die Stickstoffbeziehungen zwischen Pflanzen und Boden sich nicht durch Vorhandensein von Düngemitteln verändern, so daß sich die Wirkung der Düngemittel feststellen läßt durch Abzug der für die Parzellen ohne Stickstoff ermittelten Anteile aus dem Boden. Diese Feststellungen sind in Tabelle 18 durchgeführt worden.

Tabelle 18.
Die Stickstoffbilanz unter der Voraussetzung,
daß keine Bodenzersetzungen stattfinden.

	Stallmistdüngung		Nur künstliche Düngung Natronsalpeter + Mineralsalze	
	Kilogramm je Hektar und Jahr	Prozent	Kilogramm je Hektar und Jahr	Prozent
Die mit dem Düngemittel gegebene gesamte Stickstoffmenge . . .	108	100	37	100
Im Boden wiedergefundene Stick- stoffmenge	41	39	13	36
Von den Pflanzen aufgenommene Stickstoffmenge	32	30 ¹⁾	26	70 ¹⁾
Verlorene Stickstoffmenge . . .	34	31	— 2	— 6

Bei der Düngung mit Natronsalpeter zeigen die beiden für die Stickstoffaufnahme der Pflanzen erhaltenen Ergebnisse von 66 und 70%

¹⁾ Nach Berücksichtigung des mit dem Regen und mit dem Saatgut zugeführten Stickstoffs.

keinen großen Unterschied und die grundlegende Annahme mag hier richtig sein. Sie stimmt hingegen kaum bei der Stallmistdüngung, denn hier ist der Unterschied größer, weil die Werte für die Stickstoffaufnahme 30 bzw. 47% betragen.

Die entsprechende Berechnung ergibt für die Parzellen in Rothamsted (b) aus Stallmistdüngung folgenden Prozentsatz Stickstoff:

	Von den Pflanzen aufgenommen	Im Boden zurückgeblieben
a) Gerste (Woburn)	30	39
b) Gerste (Hoos, Parzelle 7 bis 2)		31
b) Weizen (Broadbalk, Parzelle 2)	32 ¹⁾	29
a) Weizen (Woburn)		23

Für Askov (Dänemark) sind die entsprechenden Zahlen 30 und 28 auf Lehm- bzw. Sandboden. Diese Tatsache scheint auf eine allgemein gültige Zahl hinzudeuten, die ungefähr 30% beträgt. Ohne wesentliche Beeinträchtigung des Ergebnisses kann man diese Zahlen ungefähr abrunden und die Stickstoffbilanz nach folgenden Richtlinien aufstellen:

	Auf Boden ohne stickstoffhaltige Düngung	Auf Boden mit Natronsalpeterdüngung	Auf Boden mit Stallmistdüngung
In den Pflanzen . . .	ungefähr 40%	ungefähr $\frac{2}{3}$	ungefähr $\frac{1}{3}$ bis $\frac{1}{2}$
Im Boden	—	—	ungefähr $\frac{1}{3}$
Verlust	ungefähr 60%	—	ungefähr $\frac{1}{4}$ bis $\frac{1}{3}$

Da die jährliche Zuführung von Stallmistgaben andauert, wird die Stickstoffmenge, die im Boden bleibt, von Jahr zu Jahr geringer, so daß der Verlust größer wird.

8. Die Nachwirkung stickstoffhaltiger Düngemittel.

a) Schwefelsaures Ammoniak und Natronsalpeter.

1882 wurden die Parzellen, die doppelte Menge Natronsalpeter bzw. schwefelsaures Ammoniak erhalten hatten, in zwei gleiche Hälften geteilt. Von dieser Zeit an wurde die stickstoffhaltige Düngung nur der Hälfte der Parzelle gegeben; die andere Hälfte erhielt zwar ihre übliche Mineralsalzdüngung, blieb aber ohne Stickstoffdüngung. Die stickstoffhaltige Düngung gelangte bei jeder Parzelle abwechselnd zur Anwendung. Auf diese Weise stand jedes Jahr eine Parzelle zur Verfügung, die in demselben, eine andere, die im vorhergehenden Jahr die stickstoffhaltige Düngung bekommen hatte.

In dem Parallel-Versuch in Rothamsted sinkt auf der zuletzt genannten Parzelle die Ernte immer bis zu den Erträgen einer niemals mit stickstoffhaltigen Düngemitteln behandelten Parzelle.

¹⁾ A. D. Hall: „Düngemittel und Düngungen“, 1909, S. 211.

Diese ähnlichen Versuche in Rothamsted werden gewöhnlich als Beweis für die Tatsache angeführt, daß schwefelsaures Ammoniak keine Nachwirkung zeigt. Was in der Ernte an Stickstoff nicht wieder zum Vorschein kam, wurde als verloren angesehen. In Woburn gelangte man zu abweichenden Ergebnissen, und von Anfang an hatte man den Eindruck, daß sowohl von schwefelsaurem Ammoniak als auch von Natronsalpeter ein Rest im Boden verblieb, der der darauffolgenden Ernte zugute kam. Das Weglassen der stickstoffhaltigen Düngemittel, abwechselnd, auf der einen und dann auf der anderen Hälfte der Parzelle 8 und 9, hatte nicht wie in Rothamsted ein Sinken der Ernte bis zu den Erträgen der ständig ohne Stickstoffgabe behandelten Parzelle zur Folge. Die in Woburn festgestellten Ergebnisse zeigt Tabelle 19. Während der gesamten Versuchsdauer betrug bei Gerste die durchschnittliche Nachwirkung nach Korngewicht in Kilogramm je Hektar:

	1882 bis 1906	1907 bis 1926
Natronsalpeter	507	213
Schwefelsaures Ammoniak . .	706 ¹⁾	—

Eine Erklärung für diese Ergebnisse läßt sich schwer finden. Die auf demselben Feld durchgeführten Versuche mit Gründüngung zeigen scheinbar deutlich, daß Salpeterstickstoff während des Winters nicht im Boden bleibt und doch scheint das Vorhandensein dieser Nachwirkungen unumstößlich sicher zu sein. Ein Zweifel ergab sich allerdings aus der Tatsache, daß Natronsalpeter bei Weizen keine klare Nachwirkung zeigte, wo man es eigentlich genau so erwartet hätte. Auf Gerstenparzellen ließ sich bestimmt eine länger dauernde Wirksamkeit des Natronsalpeters beobachten. In den Jahren 1928 bis 1933, in welchen die Düngung unterbrochen wurde, zeigten sich nämlich die früher mit Natronsalpeter gedüngten Parzellen trotz einer zweijährigen Brache (1927 und 1928) deutlich denjenigen überlegen, die diese Düngung nicht erhalten hatten. Schwefelsaures Ammoniak zeigte anscheinend sowohl bei Weizen- als auch bei Gerstenparzellen Nachwirkungen; die Ergebnisse dürfen hier aber nicht als sicher bewertet werden, denn es ist möglich, daß der Boden ungleich war. Merkwürdigerweise stehen die Ergebnisse von Rothamsted aus denen man schließen konnte, daß Reststickstoff nicht vorhanden sei, demgegenüber (S. 216), so daß die Frage, ob Natronsalpeter oder schwefelsaures Ammoniak als brauchbare Restbestände im Boden verbleiben, selbst heute noch nicht als gelöst zu betrachten ist. Die neuen Methoden der Feldversuche haben den großen Vorzug, daß sie eindeutige Ergebnisse erzielen und dadurch die dabei angeschnittenen Probleme befriedigend gelöst werden können.

¹⁾ 1882 bis 1891.

Tabelle 19.

Nachwirkungen von Natronsalpeter und schwefelsaurem Ammoniak bei Gerste.

	Par- zelle	1882	1883	1884	1885	1886
Erträge in den Jahren mit einer Stickstoff- düngung von 92 kg je Hektar	9	38,6	35,0	33,3	39,1	31,4
Erträge in den Jahren ohne Stickstoff		22,3	21,8	23,3	20,3	16,5
Erträge der ständig ohne Stickstoff gebliebenen Parzelle	4	13,5	16,5	19,2	11,7	10,9
Wahrscheinliche Nachwirkung		8,8	5,3	4,1	8,6	5,6

		Kornertrag in Doppelzentner je Hektar Natronsalpeter				
	Par- zelle	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906
Erträge in den Jahren mit einer Stickstoff- düngung von 92 kg je Hektar	9	35,5	28,0	23,8	25,2	27,5
Erträge in den Jahren ohne Stickstoff		20,8	17,9	17,0	14,9	14,0
Erträge der ständig ohne Stickstoff gebliebenen Parzelle	4	14,4	13,4	11,7	9,9	10,0
Wahrscheinliche Nachwirkung		6,4	4,5	5,3	5,0	4,0

Zweite Periode. Halbe Düngung.

		Kornertrag in Doppelzentner je Hektar Natronsalpeter			
	Par- zelle	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
Erträge in den Jahren mit einer Stickstoff- düngung von 46 kg je Hektar	9	16,7	13,7	12,8	10,4
Erträge in den Jahren ohne Stickstoff		9,7	10,2	8,6	6,9
Erträge der ständig ohne Stickstoff gebliebenen Parzelle	4	5,6	9,6	7,3	4,4
Wahrscheinliche Nachwirkung		4,1	0,6	1,3	2,5

		Kornertrag in Doppelzentner je Hektar Schwefelsaures Ammoniak		
	Par- zelle	1882 bis 1886	1887 bis 1891	1892 bis 1896
Erträge in den Jahren mit einer Stickstoff- düngung von 92 kg je Hektar	8	30,2	27,9	18,4
Erträge in den Jahren ohne Stickstoff		22,4	19,6	13,6
Erträge der ständig ohne Stickstoff gebliebenen Parzelle	4	14,4	13,4	11,7
Wahrscheinliche Nachwirkung		8,0	6,2	1,9

b) Stallmist-Düngung.

Einige Ergebnisse in Woburn stehen in Beziehung zu den Nachwirkungen des Stallmistes. Die erste Reihe Angaben darüber findet man vom Jahre 1882 an, als die Parzellen 10 und 11, die fünf Jahre lang 62 bzw. 124 kg Stallmist erhielten, halbiert wurden. Während der nächsten

Tabelle 20.

	Parzelle 10 a		Parzelle 11 a	
	kg/ha		kg/ha	
Die gesamte, mit der Stallmistdüngung zugeführte Stickstoffmenge.				
In fünf Jahren (1877 bis 1881)	308		616	
Der von den Pflanzen aufgenommene Stickstoff, Überschuß gegenüber den ungedüngten Parzellen.				
Direkte Wirkung in fünf Jahren (1877 bis 1881). . .	26	—	90	—
Nachwirkungen in 25 Jahren (1882 bis 1906)	224	250	470	560
Unberechneter Überschuß in Kilogramm	—	58	—	56
Unberechneter Überschuß in Prozent	—	19	—	9
Aufnahme in Prozent	—	81	—	91

25 Jahre blieb die eine Hälfte ungedüngt, dagegen erhielt die andere Hälfte dieselbe Düngung wie früher, und zwar Parzelle 10 b nur sechs Jahre lang, Parzelle 11 b hingegen bis 1906.

Die Ergebnisse sind in Tabelle 20 zusammengestellt. Parzelle 10 a hatte in den vorhergehenden fünf Jahren im ganzen ungefähr 500 dz Stallmist bekommen, der annähernd 300 kg Stickstoff enthielt, dagegen war die Parzelle 11 a mit der doppelten Menge gedüngt worden. Während der ersten fünf Jahre nach dem Fortfallen der Stallmistdüngung konnte man auf allen Parzellen — einschließlich der ungedüngten — ein deutliches Steigen der Erträge feststellen, das wahrscheinlich den günstigen Witterungsverhältnissen zuzuschreiben ist. In den folgenden, über fünf Jahre sich erstreckenden Zeiträumen sanken die Erträge der Parzellen 11 a und 10 a fortwährend, aber 25 Jahre hindurch fielen sie in dem ent-

Tabelle 21.

Durchschnittlicher Gesamtertrag bei Gerste in Doppelzentnern je Hektar.

Parzelle	1877 bis 1881	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
1 und 7	31,4	34,4	24,3	20,3	15,8	16,9	12,6	16,3	13,9	11,1
4	28,9	32,0	27,4	24,7	21,1	20,5	15,4	23,0	17,9	11,9
2	48,2	55,9	43,0	26,2	10,7	2,8	0,3	2,9	2,9	2,2
3	48,0	62,0	43,4	37,0	32,4	31,8	27,8	23,6	18,6	17,8
5	55,0	62,2	50,7	38,6	14,0	8,1	4,4	8,0	7,5	9,2
6	57,3	72,9	58,6	51,5	48,4	47,4	28,6	31,5	24,3	22,4
8 a	66,6	78,4	64,8	42,3	25,4	15,9	8,2	2,6	3,9	1,4
8 b		48,8	41,3	30,4	21,8	13,0	3,1	2,6	2,3	1,2
9 a	68,6	90,3	69,7	57,9	55,3	65,1	42,1	38,2	29,2	28,3
9 b		48,8	37,2	38,0	31,0	30,2	21,1	25,8	19,8	19,5
10 a		42,7	32,5	26,7	20,7	23,1	25,3	31,3	25,2	17,4
10 b	36,8	43,8	40,1	44,9	42,9	48,9	24,9	26,0	17,0	11,6
11 a		49,0	41,9	36,7	28,0	30,0	35,7	35,6	30,6	20,4
11 b	46,7	58,9	46,4	54,0	48,7	51,2	44,0	44,9	40,3	35,2

Tabelle 21a.
Durchschnittlicher Kornertrag bei Gerste in Doppelzentner je Hektar.

Parzelle	Düngung	1877 bis 1881	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
a) Vollständig durchgeführte Versuchsreihen je Hektar.											
1 u. 7	Ungedüngt	13,7	15,2	11,2	8,9	7,3	7,9	4,8	6,3	5,6	3,4
4 a	Mineralsalze allein	12,6	14,4	13,1	11,7	9,9	10,0	5,6	9,6	7,3	4,4
2 a	Schwefelsaures Ammoniak (bis 1906 46 kg N, von da an 23 kg N)	21,2	21,8	20,4	12,0	4,1	0,4	0,0	1,2	0,8	1,1
3 a	Natronsalpeter (46 kg N)	20,0	26,9	18,9	16,5	14,5	14,1	10,6	8,0	7,4	6,0
3 b	Natronsalpeter (23 kg N)	—	—	—	—	—	—	8,7	8,1	5,3	4,7
5 a	Mineralsalze wie Parzelle 4 a und schwefel- saures Ammoniak wie Parzelle 2 a	23,3	28,2	24,1	17,8	6,2	2,6	1,7	2,0	2,7	3,3
6	Mineralsalze wie Parzelle 4 a und Natron- salpeter (46 kg N bis 1906, von da an 23 kg N)	23,8	30,8	26,0	22,4	22,1	20,6	11,7	12,2	10,6	8,7
8 a u.	Mineralsalze wie Parzelle 4 a und schwefel- saures Ammoniak (92 kg N bis 1906, von da an 46 kg N), ein Jahr um das andere abwechselnd der Hälfte 8 a und der Hälfte 8 b gegeben.	27,6	33,3	27,9	18,4	12,1	6,2	3,4	1,0	1,6	0,6
8 b	Schwefelsaures Ammoniak zugeführt	—	22,4	19,6	13,6	10,0	6,1	1,3	0,7	0,7	0,4
9 a u.	Mineralsalze wie Parzelle 4 a und Natronsalp- eter (92 kg N bis 1906, von da an 46 kg N), ein Jahr um das andere abwechselnd der Hälfte 9 a und der Hälfte 9 b gegeben.	27,0	35,5	28,0	23,8	25,2	27,2	16,7	13,7	12,8	10,4
9 b	Natronsalpeter zugeführt	—	20,8	17,9	17,0	14,9	14,0	9,7	10,2	8,6	6,9
10 a	1882 bis 1906 ungedüngt (mit Ausnahme von Rapskuchen wie Parzelle 10 b, 1889); 1907 bis 1926 1,5 dz Superphosphat und Natron- salpeter (23 kg N)	—	18,3	15,7	12,2	9,6	11,0	9,6	12,5	11,0	6,0
10 b	1877 bis 1887 Stallmist (62 kg N), 1888 unge- düngt, 1889 bis 1906 Rapskuchen (46 kg N im Jahre 1889, 92 kg N später), 1907 bis 1926 Rapskuchen (23 kg N)	15,8	19,4	25,0	20,3	19,0	22,2	10,7	10,5	6,7	3,5
11 a	1882 bis 1906 ungedüngt, 1907 bis 1926 schwe- felsaures Kali, Natronsalpeter (23 kg N)	—	22,5	19,5	16,4	12,8	11,2	11,1	13,8	13,2	7,6
11 b	Stallmistdüngung (entsprechend 174 kg N bis 1906, von da an 91 kg N)	21,1	26,6	21,5	24,5	21,7	23,6	19,1	18,0	17,0	13,8
b) Abgeänderte Versuchsreihen je Hektar.											
4 b	Wie Parzelle 4 a mit 25 dz Kalk im Jahre 1915	—	—	—	—	—	—	10,8	8,0	5,4	—
2 b	Wie Parzelle 2 a mit 50 dz Kalk, 1897 u. 1912	—	—	—	—	14,2	12,1	6,0	11,4	5,4	4,8
2 aa	Wie Parzelle 2 a mit 6 dz Kalk im Jahre 1905, 1909, 1910 und 1912 und 12 dz 1923	—	—	—	—	—	5,2	3,3	4,9	3,4	2,8
2 bb	Wie Parzelle 2 a mit 50 dz Kalk im Jahre 1897 und 1905	—	—	—	—	—	21,4	9,3	8,4	4,7	5,2
3 aa	Wie Parzelle 3 a mit 50 dz Kalk im Jahre 1921	—	—	—	—	—	—	—	—	—	4,7
3 bb	Wie Parzelle 3 b mit 50 dz Kalk im Jahre 1921	—	—	—	—	—	—	—	—	—	4,5
5 b	Wie Parzelle 5 a mit 50 dz Kalk im Jahre 1897 und 1912	—	—	—	—	20,8	21,4	10,2	14,8	8,5	7,0
5 aa	Wie Parzelle 5 a mit 25 dz Kalk im Jahre 1905 und 1916	—	—	—	—	—	12,0	14,1	9,1	10,6	8,4
8 aa u.	Wie Parzelle 8 a und 8 b mit 50 dz Kalk im Jahre 1897 und 1912	—	—	—	—	—	—	—	—	—	—
8 bb	Schwefelsaures Ammoniak zugeführt	—	—	—	—	25,0	22,7	12,0	15,8	10,7	7,6
	Schwefelsaures Ammoniak weggelassen	—	—	—	—	17,5	17,8	7,1	11,4	7,4	5,7

sprechenden Verhältnis zueinander und zu der ungedüngten Parzelle, und die Unterschiede in dem Ertrage stimmten mit den Unterschieden in der ursprünglichen Düngung in jeder Beziehung überein. Leider wurde der Versuch 1906 abgebrochen. Es wäre sehr interessant gewesen, zu erfahren, wie lange die Erträge dieser beiden Parzellen die Ernte der ungedüngten Parzellen übertroffen hätten und ob sie sich — wenn dies der Fall wäre — einmal stabilisieren würden.

Die Ergebnisse werden später besprochen, inzwischen muß gesagt werden, daß das gesamte Ergebnis nur durch die Bodenungleichmäßigkeiten beeinflusst sein kann.

Tabelle 21b.

Durchschnittliche Kornerträge bei Gerste in Prozenten des Gesamtertrages.

Parzellen	Erste Periode 1877 bis 1891			Zweite Periode 1892 bis 1906			Dritte Periode 1907 bis 1926			
	1877 bis 1881	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
1	44	45	45	42	44	46	38	39	39	32
7	43	44	47	45	48	48	38	39	43	30
4	43	45	49	47	46	49	37	42	40	37
2 ¹⁾	43	44	47	45	42	14	0 ²⁾	41	40	48
3 a	41	44	44	44	45	44	38	33	39	34
5 ¹⁾	42	46	47	45	41	32	41	26	39	36
6	41	43	44	44	45	43	40	39	43	39
8 a mit Stickstoff	41	43	43	44	47	39	42	41	39	41
8 b ohne Stickstoff ¹⁾		46	48	44	46	46	44	30	40	37
9 a mit Stickstoff ¹⁾	39	40	40	42	46	42	39	36	43	37
9 b ohne Stickstoff		48	49	44	48	46	46	40	43	36
10 a		46	45	45	46	17	38	39	44	34
10 b	42	46	45	45	44	45	42	40	39	30
11 a		46	46	45	45	47	39	38	43	38
11 b	45	46	47	45	45	46	45	40	43	39

9. Korn- und Strohausbildung.

Das Verhältnis zwischen Gesamtertrag und Kornertrag.

Das durchschnittliche Verhältnis zwischen Kornertrag und Gesamtertrag ist innerhalb der ersten 25 Jahre und bei den meisten Parzellen sogar innerhalb der ersten 30 Jahre für jeden 5 Jahre umfassenden Zeitraum (Tabellen 21, 21a, 21b) im wesentlichen das gleiche, unbeeinflusst von der jeweiligen Düngung. Ob die Gerste auf armem, ungedüngtem Boden, dessen Fruchtbarkeit stetig abnahm, oder auf einem durch Stallmistdüngung oder künstliche Volldüngung angereicherten Boden wuchs, es entfiel immer annähernd der gleiche Anteil — etwa 45 % — auf den Kornertrag. Das Verhältnis ist etwas niedriger für die Pflanzen, die mit Natronsalpeter gedüngt waren, als für die, die entweder keinen Stickstoff oder schwefelsaures Ammoniak erhalten hatten; es ist am niedrigsten bei Parzelle 9, die die doppelte Menge Düngemittel erhalten hatte. So gab

¹⁾ Die Jahre, in welchen Korn- und Strohmenge nicht eingetragen war, sind bei diesen durchschnittlichen Zusammenstellungen unberücksichtigt geblieben.

Hinterkorn wurde bis 1896 zum Stroh gerechnet, von da ab zum Korn.

²⁾ Von 1907 bis 1911 keine Erträge.

Natronsalpeter mehr Stroh als schwefelsaures Ammoniak und steigerte bei Anwendung in großen Mengen mehr den Strohertrag als den Kornertrag. Von einem Jahr zum andern veränderte sich das Verhältnis etwas; in den Jahren mit guten Ernten sank das Verhältnis bei den mit Natronsalpeter und Stallmist behandelten Parzellen immer, d. h. der Strohertrag nahm mehr zu als der Kornertrag; aber umgekehrt wurden bei den ohne Stickstoff behandelten Parzellen (1,7 und 4) die Kornerträge mehr gesteigert als die Stroherträge, und für jeden Doppelzentner je Hektar Zunahme im Gesamtertrag stieg das Verhältnis um ungefähr 0,6 %.

Nach den ersten 25 Jahren begann das Verhältnis für die mit schwefelsaurem Ammoniak gedüngten Pflanzen zu sinken und nach den ersten 30 Jahren fiel auch das Verhältnis bei den anderen Parzellen, so daß der durchschnittliche Anteil Kornertrag am Gesamtertrag nur noch 39 % betrug. Wie schon oben erwähnt, verschlechterten sich auch die Ernten.

Die Ursache hierfür mag zum Teil an einem Wechsel in der Verteilung der Trockensubstanz innerhalb der Pflanzen liegen, zum anderen Teil in der Schwierigkeit, in Jahren mit geringen Erträgen viel Korn zu bilden. Die Berechnung des Verhältnisses hat bei kleinen Pflanzen weniger Zweck als bei großen, denn in dem gleichen Maße, wie die Pflanzen abnehmen, wächst auch das Unkraut und trägt so mehr und mehr zu dem „Gesamt-Ertrag“ bei.

Während der Versuchszeit, die sich über 50 Jahre erstreckte, war mit den Gerstensorten öfters gewechselt worden und diese Tatsache schien auch das Verhältnis von Kornertrag zu Gesamtertrag in gewisser Hinsicht zu beeinflussen. Zuerst wurde der Versuch für eine Zeitspanne von sechs Jahren mit der Gerstensorte „Chevalier“ angesetzt, die ein Verhältnis von 41,8 zwischen Korn- und Gesamtertrag ergab. Danach wurde die Sorte „Golden Melon“ genommen; nachdem sie 14 Jahre im Versuch gestanden hatte, wurde sie aus dem Handel gezogen. Während dieser Periode änderte sich das Verhältnis nur wenig und brachte trotz beträchtlicher Abnahme der Ernten einen Durchschnittswert von rund 45 % Kornanteil. Von 1904 bis 1908 wurde „Chevalier“ wieder benutzt: diese Gerste senkte jetzt aber das Verhältnis nicht auf den früher bei „Chevalier“ ermittelten Wert, sondern hatte noch ein durchschnittliches Verhältnis von 45,3. Daraufhin wurde vier Jahre mit „Goldthorpe“ gearbeitet, bei welcher geringere Erträge und ein niedrigeres Verhältnis festgestellt wurde. „Goldthorpe“ wurde dann durch die Sorte „Chevalier“ ersetzt, mit der die Versuche sieben Jahre lang (1913 bis 1919) durchgeführt wurden. Obgleich die Ernte um die Hälfte gesunken war, stieg das Verhältnis von Kornertrag zu Gesamtertrag auf 41,7, den gleichen Wert wie in der ersten Periode. Vom Jahre 1922 an setzte ein Absinken der Ernten ein und das Verhältnis zwischen Korn- und Gesamtertrag wurde niedriger. Diese Erscheinungen kann man wohl kaum der von dieser Zeit an verwendeten Gerstensorte „Plumage Archer“ zuschreiben, sondern sie werden durch das immer stärker werdende Unkraut verursacht. Die Werte sind in Tabelle 22 dargestellt.

Die für jede Gerstensorte bis genau zu dem Zeitpunkt des Sinkens der Erträge festgestellte geringe Abweichung in dem Verhältnis zeigt, daß bei der Besprechung allgemeiner Versuchsergebnisse Kornertrag und Gesamtertrag ohne irgendwelche Beeinträchtigung gleichgesetzt werden können.

Tabelle 22.

**Gesamtertrag und Kornertrag und das Verhältnis
zwischen Kornertrag und Gesamtertrag unter Berücksichtigung
der verschiedenen, für diesen Versuch benutzten Gerstensorten.**

Zeitraum	Sorte	Mittelwert (des Verhältnisses ¹⁾) Kornertrag zu Gesamtertrag	Durchschnittlicher Wert der Erträge in Doppelzentnern je Hektar	
			Kornertrag	Gesamtertrag
1877 bis 1880	Nicht eingetragen	41,2	18,6	45,2
1881 bis 1882	Chevalier	43,5	25,1	57,7
1883 bis 1887	Golden Melon	45,1	22,6	50,1
1890 bis 1894	Golden Melon	45,4	20,8	45,8
1895 bis 1898	Golden Melon	42,1	12,8	30,4
1899 bis 1900	Golden Melon	49,0	16,8	34,3
1904 bis 1908	Chevalier	45,3	13,4	29,7
1909 bis 1912	Goldthorpe	36,8	8,7	23,7
1913 bis 1919	Chevalier	41,7	10,3	24,7
1922 bis 1926	Plumage Archer	35,5	6,2	17,6

10. Die jährlichen Ertragsschwankungen.

Es zeigt sich immer wieder als außerordentlich störend, daß die Feldversuche niemals unter genau denselben Bedingungen wiederholt werden können, und die Ergebnisse des einen Jahres infolgedessen niemals mit denjenigen des anderen Jahres übereinstimmen. Zur Vermeidung dieser Schwierigkeit hatte man in Rothamsted von jeher die folgende Methode gewählt: Man wiederholte den Versuch 20 Jahre hintereinander auf demselben Boden; die Mittelwerte von fünf oder zehn aufeinanderfolgenden Jahren ergaben dann die Grundlage für die Prüfung. Witterungseinflüsse wurden insofern berücksichtigt, daß man die Ergebnisse eines ausgesprochen trockenen denjenigen eines besonders feuchten Jahres gegenüberstellte und annahm, daß die Differenz durch Unterschiede in der Niederschlagsmenge bedingt sei. Abgesehen von dem unverkennbaren Nachteil, daß es lange dauert, bis man in den Besitz der Ergebnisse gelangt, wirkt es weiterhin ungünstig, daß man nur aus der Beobachtung wenig sicheres Beweismaterial erhalten kann.

Die Ursachen für den verschiedenen Ausfall der Erträge sind zum Teil wohl bekannt. Der Hauptgrund liegt in der Witterung während der

¹⁾ Nach 1891 blieb Parzelle 2 unberücksichtigt.

Anmerkung: In den oben nicht angeführten Jahren wurden folgende Sorten angebaut: 1888 nicht bekannt. 1889 Webb's Melon. 1901 Standwell. 1902 Hallett's Pedigree. 1908 Standwell. 1920 Plumage. 1921 Chevalier.

Vegetationsperiode. Einige Sommer zeigen deutlich einen günstigen Einfluß auf das Wachstum der Pflanzen, andere dagegen einen besonders schlechten. Es ist noch keinem gelungen, eine gute Wachstumsperiode durch meteorologische Angaben festzulegen. Messungen der Niederschlagsmenge, der Temperatur, der Sonnenscheinstunden und anderer meteorologischer Faktoren werden regelmäßig durchgeführt; aber sie können alle überhaupt keine genauen Wachstumsbedingungen angeben. Es sind zwar einige Richtlinien für diese Wechselbeziehungen ausgearbeitet worden, wie z. B. die Kurven von Shaw, die den ungünstigen Einfluß eines nassen Winters auf die Weizenernte in den östlichen Ländern zeigt, doch nutzen die meteorologischen Angaben im allgemeinen dem Landwirt nicht sehr viel. Die vorliegende Arbeit bringt dies deutlich zum Ausdruck und zeigt das Bedürfnis nach weit eingehenderen Forschungen auf dem Gebiet der Agrar-Meteorologie.

Ein anderer Grund für die Ertragsschwankungen ist die Veränderung, die im Boden vor sich geht und bedingt wird durch seine Behandlung, einschließlich der Wirkungen der Düngemittel, der Pflanzen, der Bearbeitung und der Witterung. Bei fortlaufend gleichmäßiger Bodenbearbeitung, wie sie die Versuche mit Dauer-Gersten- und -Weizenanbau darstellen, machen sich diese Einflüsse naturgemäß am meisten bemerkbar; sie dauern von Jahr zu Jahr an und unterscheiden sich dadurch von der durch die Witterung verursachten Beeinflussung des Wachstums, die mit der Ernte abgeschlossen ist und auf das nächste Jahr nicht die geringste Wirkung ausübt.

Eine dritte Ursache für die Ertragsschwankungen bilden Unkraut und Krankheiten ansteckender oder nichtansteckender Natur, Faktoren, die von einem Jahr auf das nächste übertragen werden und zuweilen einen geringen, zuweilen einen stärkeren Einfluß zeigen. Diese Veränderungen sind zwar fortlaufend aber nicht beständig zunehmend wie die oben erwähnten Änderungen des Bodens.

In den letzten Jahren hat R. A. Fisher in Rothamsted statistische Methoden zur Unterscheidung dieser drei für die Ertragsschwankungen verantwortlichen Faktoren ausgearbeitet. Stellt man für alle 50 Jahre die Erträge einer Parzelle graphisch dar, so bilden diese eine Reihe von Punkten, die auf eine ziemlich große Fläche verstreut sind; verbindet man die Punkte untereinander, erhält man eine Zickzack-Linie. Man kann durch diese Punkte eine glatte Kurve ziehen, die die allgemeine Richtung für die Verteilung dieser Punkte angibt. Diese Kurve läßt sich in zwei Komponenten zerlegen: in eine gerade Linie, die die fortlaufende gleichmäßige Richtung zum Ausdruck bringt und in eine nicht geradlinige Komponente, die die ununterbrochenen, aber verschiedenartigen Wirkungen darstellt. Die Abstände der einzelnen Punkte von der Kurve geben die nur in einer einzigen Vegetationsperiode zum Ausdruck gekommenen Wirkungen an; über die vorher oder nachher beobachteten Folgen geben sie keinen Aufschluß.

Durch diese statistische Darlegung erhält man nur über die Wirkungen, nicht aber über die Ursachen Auskunft; aber es erscheint ganz vernünftig,

diese Wirkungen mit den oben erwähnten verschiedenen Ursachen in Verbindung zu bringen und anzunehmen, daß die Linien-Komponente der glatten Kurve — die fortlaufend gleichmäßig abwärtsführende Richtung — die Bodenverschlechterung darstellt, und daß der nicht geradlinige Teil der Komponente, der zum Teil aufwärts, zum Teil abwärts führt, die ständigen aber doch verschiedenartigen Faktoren, wie Unkraut oder Beeinträchtigung durch Krankheit, zum Ausdruck bringt; während die anscheinend unregelmäßig und von der Kurve völlig verstreut liegenden Ertragswerte den Einfluß der Witterung zeigen; jede Vegetationsperiode weicht von derjenigen des vorausgegangenen Jahres ab und steht in keinem Zusammenhang mit ihr. Diese Verbindung läßt sich natürlich nicht genau durchführen: Außer der Bodenverschlechterung haben auch noch andere Faktoren eine „geradlinige“ Komponente und erscheinen dadurch als Verschlechterung, während andere Faktoren, die die Bodenverschlechterung bedingen, keine gleichmäßigen, fortdauernden Wirkungen zeigen. Der Entzug des Kalkes auf der mit schwefelsaurem Ammoniak behandelten Parzelle ging wahrscheinlich ununterbrochen und gleichmäßig vor sich, aber seine Wirkung war nicht so; sie setzte plötzlich ein und war dann so stark, daß innerhalb weniger Jahre die Erträge soweit herabsanken, daß sie kaum noch auf einen tieferen Stand kommen konnten. Die jährlich auftretenden Schwankungen sind sicherlich nicht alle unmittelbar durch die Witterung veranlaßt, auch andere Faktoren spielen mit, z. B. Vogel- und Insektenplage, vereinzelt auftretende Krankheiten sowie die jährlich zu beobachtenden Beeinträchtigungen durch Unkraut.

Die drei Arten dieser Faktoren sind in Tabelle 23 nach ihrer relativen Wichtigkeit geordnet; hier ist ein Vergleich mit Rothamsted und den Spalten der Abbildungen 4a und 4b, die sich wie folgt zusammenfassen lassen, durchgeführt.

Düngung	Die Abweichungen in Prozenten ¹⁾	
	Alle Ursachen	Jahres-Ursachen
Stallmist-Düngung	8 18—29	5—6
Volldüngung mit Natronsalpeter		12
Keine Stallmistdüngung		
Unvollständige Düngungen		
Nur Mineralsalze	18	15
Nur Natronsalpeter	17	8
Saure Parzellen (2a und 5a)	50—63	7—10

Die Rubriken stellen die Schwankungen in Prozenten dar; sie sind die Quadrate der Standard-Abweichung in Prozenten. Man berechnet die Schwankungen deshalb nach Prozenten, weil man diese dann sofort addieren kann, um die gesamte Abweichung festzustellen, die sich dagegen beim Arbeiten mit der Standard-Abweichung nicht so leicht berechnen läßt.

¹⁾
$$= \frac{\text{Abweichung} \times 100}{\text{Durchschnittlicher Ertrag}}$$

Die „prozentische Abweichung“ ist der beste Maßstab für die von Jahr zu Jahr beobachtete Schwankung im Ertrage¹⁾. Die geringsten Abweichungen zeigen die Parzellen mit Stallmist- und mit Volldüngung; die Veränderungen erhöhen sich bei Parzellen mit unvollständiger Düngung und noch mehr bei solchen Parzellen, die sauer geworden sind. Bei den Parzellen mit Volldüngung gehen die jährlich festgestellten Abweichungen größtenteils auf das Konto der Gesamtänderungen, während auf den Par-

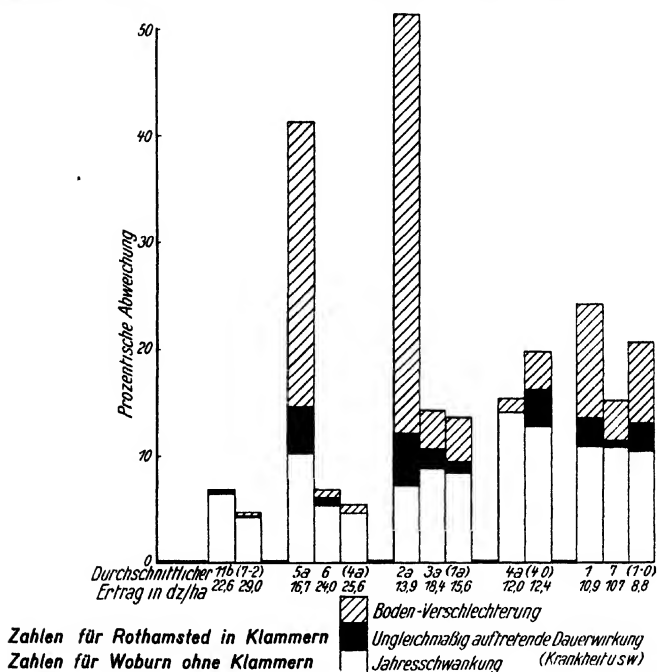


Abb. 1a.

Eine Gegenüberstellung der Ursachen, die in Woburn während einer 30jährigen Beobachtungszeit, auf dem Hoos-Feld in Rothamsted in einer 70jährigen Versuchszeit zu einer Schwankung der Gerstenerträge führten.

zellen ohne Mineraldüngung (auf der ungedüngten und auf der nur mit Natronsalpeter behandelten) die Hälfte und auf den sauren Parzellen weniger als ein Fünftel der prozentischen Abweichungen den Gesamtschwankungen zuzuschreiben sind. Da die Volldüngung eine Maßnahme ist, die allgemein auch dem praktischen Anbau empfohlen wird, haben diese Jahreseinflüsse eine große praktische Bedeutung.

11. Witterungseinflüsse und andere Jahreseinflüsse.

Die oben gebrachten Abbildungen zeigen, daß der Prozentsatz der jährlich festgestellten Einflüsse bei allen Stickstoffparzellen etwa der

¹⁾ Die statistische Darstellung ist besprochen von Cochran in dem Buch „50 Jahre Feldversuche der Versuchsstation Woburn. Von E. John Russell und J. A. Voelcker. Mit einem statistischen Bericht von W. G. Cochran.“

gleiche (6 oder 8 %) ist, sich aber ungefähr verdoppelt bei den Parzellen, die keine Stickstoffdüngung erhalten haben. Düngungen mit Natronsalpeter, schwefelsaurem Ammoniak und Stallmist halten die Erträge von Jahr zu Jahr auf der gleichen Höhe und alle drei scheinen gleich wirksam zu sein. Die Parzellen in Rothamsted brachten dasselbe Ergebnis, und die hieraus abgeleitete praktische Folgerung ist die, daß eine gute Düngungsweise nicht nur das Wachstum der Pflanzen fördert, sondern auch auf dieser

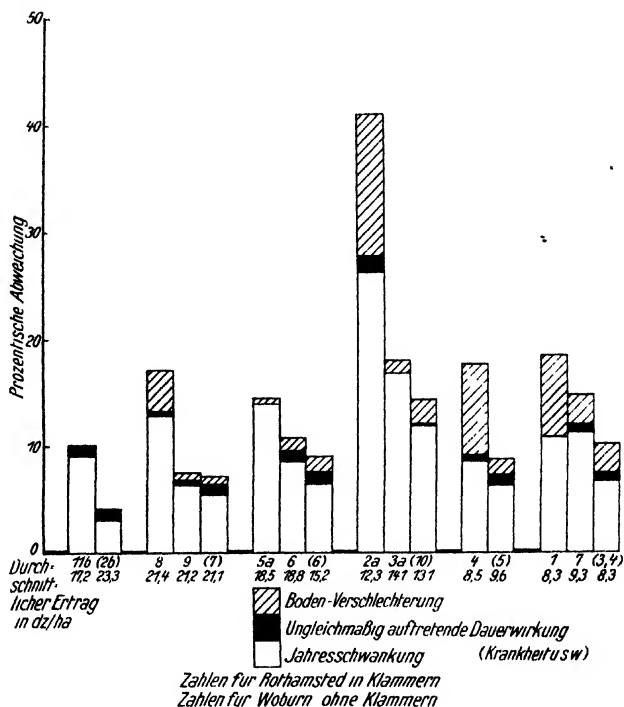


Abb. 4b

Eine Gegenüberstellung der Ursachen, die in Woburn während einer 30jährigen Beobachtungszeit (1877 bis 1906) und auf dem Broadbalk-Feld in Rothamsted in einer Versuchszeit von 67 Jahren zu einer Schwankung der Weizen erträge führten.

Höhe erhält, so daß diese Pflanzen unter der Witterung viel weniger leiden als die Pflanzen einer schlecht gedüngten Parzelle. Auf der anderen Seite hat eine fehlerhafte Düngungsart, besonders wenn Stickstoff nicht in genügender Menge verwendet wird, geringere und abweichendere Erträge zur Folge, die von einer schlechten Vegetationsperiode erheblich beeinträchtigt werden. Die schlechtesten Ergebnisse kann man auf saurem Boden beobachten, eine Tatsache, die sich nicht mit der durch die Vegetationsperiode hervorgerufenen Veränderung, sondern mit dem fortdauernden schädigenden Einfluß des Bodens erklären läßt.

Die Ursachen der Jahres-Schwankungen umfassen sowohl alle Witterungseinflüsse als auch zufällige Schäden wie Vogel- und Insekten-

fraß, parasitäre Krankheiten, Fehler beim Anbau usw. R. A. Fisher hat Methoden ausgearbeitet, um festzustellen, welche Wirkung jedem einzelnen, meßbaren Faktor zuzuschreiben ist. Diese Methoden hat man benutzt, um die Wirkungen der Niederschläge zu untersuchen. Die Kurven der Abbildung 5 stellen die geradlinigen Wirkungen dar, d. h. diejenigen, die unmittelbar von der Menge der Niederschläge abhängen und in dem gleichen Verhältnis wie die Niederschläge steigen oder sinken, stärker oder schwächer

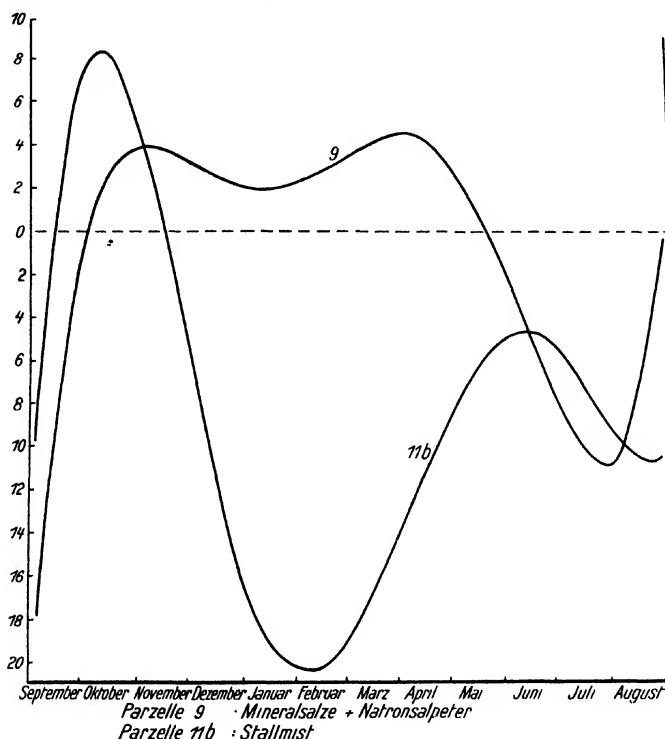


Abb. 5.

Die Wirkung von 25 mm Niederschlägen über dem Durchschnitt auf Steigerung oder Senkung der Kornerträge bei Gerste, berechnet in Doppelzentner je Hektar.

werden. Auf dem Versuchsfeld Broadbalk in Rothamsted stellen diese geradlinigen Wirkungen bei Weizen einen großen Teil der gesamten Wirkung der Niederschläge dar, ein Ergebnis, das man bei Gerste und Mangold nicht beobachten konnte. Auch in Woburn machten sie sowohl bei Weizen als auch bei Gerste nur einen kleinen Teil der gesamten Wirkung der Regenmenge aus (Tabelle 24) und sind nur gelegentlich rechnerisch gesichert. Eine Begründung dafür liegt möglicherweise darin, daß die Methode dann unbrauchbar ist, wenn sich die wirkliche Niederschlagsmenge in irgendeinem der angegebenen Zeiträume an oder in der Nähe eines Optimums befindet. Die Kurve zeigt, daß für die gradlinigen Wirkungen weitere Niederschläge jeder Art, wann sie auch immer einsetzen

Tabelle 23.

Durchschnittlicher Kornenertrag und jährliche Abweichung.

Düngungsart	Woburn (1877 bis 1906)						Rothamsted (1852 bis 1921)					
	Parzelle	Durchschnittlicher Ertrag Doppelzentner je Hektar ¹⁾	Abweichung, berechnet aus			Parzelle	Durchschnittlicher Ertrag Doppelzentner je Hektar ¹⁾	Abweichung, berechnet aus				
			allen Ursachen	Jahres-Ursachen allein	Prozent der Abweichung			allen Ursachen	Jahres-Ursachen allein	Prozent der Abweichung		
Stallmistdüngung	11 B	22,4	5,9	8	5,6	6	7-2	26,9	5,9	4,5	5,6	4
Vollständige Mineralsalzdüngung und Natronsalpeter	6	23,8	6,2	8	5,5	5	4 A A	17,1	5,7	—	4,9	—
Vollständige Mineralsalzdüngung, kein Stickstoff	4	11,9	4,7	18,5	4,5	14,5	4-0	11,5	5,2	22,0	4,1	12,5
Nur Natronsalpeter	3 A	18,2	6,9	17	5,4	8,5	1-A	14,5	5,4	—	4,2	—
Ungedüngt	1	10,8	5,3	29	3,7	12	1-0	8,2	3,7	22,5	2,6	10,5
Ungedüngt,	7	10,7	4,1	18,5	3,5	11	6-1	9,0	4,1	—	2,8	—
Vollständige Mineralsalzdüngung und schwefelsaures Ammoniak	5 A	16,6	—	50	5,0	10	4-A	23,5	—	6,0	—	5,0
Schwefelsaures Ammoniak allein	2 A	13,8	—	62	4,1	7	1-A	14,5	—	15,0	—	8,0

¹⁾ Die mittleren Erträge von Rothamsted und Woburn lassen sich nicht genau miteinander vergleichen, da verschiedene Jahresperioden durchschnittlich berechnet sind; auch die Menge der Düngergabe (Natronsalpeter) zeigt Abweichungen (48 kg je Hektar Stickstoff in Rothamsted und ungefähr 200 dz je Hektar in Woburn).

²⁾ Standard-Fehler der Erträge eines einzelnen Jahres, berechnet aus der Gesamt-Abweichung. Infolge des großen Unterschiedes, der bezüglich der Länge der Versuchszeiten in Rothamsted und Woburn bestand, lassen sich für die beiden Stationen die Zahlen der gesamten Änderung, die auch langfristige Wirkungen umfaßt, nicht miteinander vergleichen. Andererseits können die Zahlen in der nächsten Rubrik, da sie sich auf Jahres-Ursachen beziehen, miteinander in Vergleich gebracht werden.

³⁾ Standard-Fehler der Erträge ohne Berücksichtigung der Wirkung der Verschlechterung und der langsam vor sich gehenden Veränderungen.

mögen, gewöhnlich schädlich sind¹⁾; die durchschnittliche Niederschlagsmenge in Woburn (600 mm im Jahr) scheint für den Bedarf der dort wachsenden Gerste sehr reichlich zu sein und jede weitere Niederschlagsmenge ist wahrscheinlich mehr von Schaden als von Nutzen. Der schädigende Einfluß kommt besonders schon in den Frühjahrsmonaten Februar und März deutlich zum Ausdruck; in diesen Monaten ist eine durchschnittliche Regenmenge von 3,1 Zoll (= 78,74 mm) schon mehr als genug, und weitere Niederschläge zeigen deutlich eine schädliche Wirkung.

Tabelle 24.

Der Prozentsatz der Abweichung, berechnet aus den geradlinigen Wirkungen und aus der Verteilung der Niederschlagsmengen.

Parzellen	Niederschlagsmenge in 6 Monaten		Niederschlagsmenge in 12 Monaten	
	Prozentische Abweichung, berechnet aus der		Prozentische Abweichung, berechnet aus der	
	Gesamtmenge der Niederschläge	Verteilung der Regenmenge	Gesamtmenge der Niederschläge	Verteilung der Regenmenge
Serien ohne Stickstoff				
1	6	5	22	1
7	16	—	11	10
4 a	14	—	12	14
Serien mit schwefelsaurem Ammoniak				
2 a	—	5	—	—
5 a	—	17	—	—
8	—	4	—	—
Serien mit Natronsalpeter				
3 a	6	—	4	—
6	7	—	5	—
9	1	0,3	—	—
Stallmist-Serie				
11 b	6	6	6	—

Die Natronsalpeter-Parzellen leiden darunter weniger, ein Beweis, daß das Nitrat den schädlichen Einfluß des Frühjahrsregens auf die Pflanzen vermindert. Die Abschwächung der schädigenden Wirkung der Frühjahrsniederschläge bei Anwendung steigender Stickstoffgaben zeigen die nachstehenden Zahlen²⁾, die man aus den Kurven ermittelt hat.

¹⁾ Selbst die Niederschläge im Winter (September bis Februar) beeinflussen die Ergebnisse, wie aus dem Unterschied zwischen den Kurven für 6 und für 12 Monate ersichtlich ist.

²⁾ Die Zahlen haben nur relativen, nicht absoluten Wert, sie geben nur die Richtung oder die Unterschiede an; aber streng genommen, können sie nicht von der Kurve getrennt werden. Sie haben nur dann Wert, wenn die Niederschläge des ganzen Jahres berücksichtigt werden und wenn die durchschnittliche Höhe der Regenmenge außer für die in Frage kommenden Zeiträume auch für andere festgestellt wird. Die bei der Messung der Niederschlagsmenge in den Monaten Februar bis August, d. h. in der Vegetationsperiode, erhaltenen Kurven lassen ähnliche Folgerungen zu, aber die tatsächlichen Zahlen sind gegenüber diesen eher höher. Diese Zahlen können normalerweise nicht als Unterlagen zum Bestimmen der Ernteaussichten benutzt werden.

Tabelle 25.
Steigende Stickstoffgaben.

Stickstoff als Natronsalpeter gegeben Kilogramm je Hektar	Parzellen	Durch- schnittlicher Kornertrag Kilogramm je Hektar 1877 bis 1906	Die nach Kilogramm je Hektar berechnete Abweichung im Kornertrag, verursacht durch 25 mm weitere Nieder- schläge im	
			Februar bis März	Juli
0	1, 7, 4	1112	— 128	— 70
46	6	2377	— 70	— 111
46	3	1823	— 82	— 82
92	9	2720	+ 29	— 87
Stickstoff als Stall- mist gegeben 123	11 b	2225	— 175	— 64

Auch in Rothamsted erhielt man ein ähnliches Ergebnis.

Die Stallmist-Parzelle (11 b) unterschied sich sehr deutlich von den Parzellen mit künstlichen Düngemitteln. Trotz der starken Steigerung der Stickstoffgabe durch Verwendung der Stallmistdüngung hatten die Frühjahrsniederschläge eine starke Ertragsdrückung zur Folge, die völlig übereinstimmte mit der auf Parzellen ohne Stickstoffdüngung beobachteten Verminderung. Andererseits wirkt sich hier anscheinend weiterer Regen im Oktober günstig aus, eine Beobachtung, die man bei anderen Parzellen nicht machen konnte.

Da Stallmistdüngung in mancher Beziehung den Charakter eines stickstoffhaltigen Düngemittels zeigt, fällt die Verschiedenartigkeit hinsichtlich der Reaktion auf Niederschlagsmengen umso mehr auf. Man muß bedenken, daß diese beiden Düngemittel nach ganz verschiedenen Methoden zur Anwendung kommen. Mit Natronsalpeter wird im Spätmärz oder April gedüngt, nachdem die Februarniederschläge vorüber sind, von welchen diese Düngung also noch garnicht beeinträchtigt wird, so daß sie zur Überwindung schädigender Einflüsse vollverfügbar ist. Stallmist dagegen kommt als Kopfdüngung im Januar zur Anwendung, so daß er der auslaugenden Wirkung der Februar-Niederschläge ausgesetzt ist und dadurch ein Teil der für die Düngung wertvollen Bestandteile ausgewaschen wird.

Es wurde auch noch eine andere statistische Untersuchungsmethode benutzt, die nicht nur mit den sogenannten geradlinigen Wirkungen arbeitet, sondern mehr mit Komplexwirkungen (mit quadratischen und kubischen Darstellungen). Nach dieser Methode scheinen März und April für jeden Überschuß an Niederschlägen die ungünstigsten Monate zu sein; selbst die durchschnittliche Niederschlagsmenge dieser beiden Monate (83 mm) ist zu hoch und bessere Ernten werden durch geringere Niederschlagsmengen erzielt.

Die Kurven (z. B. Abb. 6) zeigen aber auch, daß zu wenig Regen schädlich ist und daß man die besten Ergebnisse bei einer Niederschlagsmenge von ungefähr 37,5 bis 50,0 mm in diesen beiden Monaten feststellen kann. Die Ergebnisse sind in Tabelle 26 zusammengefaßt.

Tabelle 26.

Die durch günstig bzw. ungünstig wirkende Niederschlagsmengen hervorgerufenen Ertragssteigerungen bzw. Ertragsminderungen in Doppelzentnern je Hektar

	Parzellen	Mittlere Erträge in 29 Jahren 1878 bis 1906 Korntrag in Doppelzentnern je Hektar	Günstig wirkende Niederschlagsmenge im März bis April (87,5 bis 50,0 mm) Ertragssteigerung	Ungünstig wirkende Niederschlagsmenge im März bis April (über 100,0 mm) Ertragsminderung
Ungedüngt	1,7	10,7	1,5 bis 2,9	1,2 bis 1,8
Nur Mineralsalze	1	12,0	4,7	2,3
Mineralsalze + Natronsalpeter	6,9	26,0	4,1	2,9
Mineralsalze				
+ schwefelsaures Ammoniak	5,8	18,7	2,3 bis 3,5	1,7 bis 2,3
Nur Natronsalpeter	3	18,6	6,4	2,3
Nur schwefelsaures Ammoniak	2	13,6	2,1	1,2
Stallmistdüngung	11 b	23,4	2,6	4,1

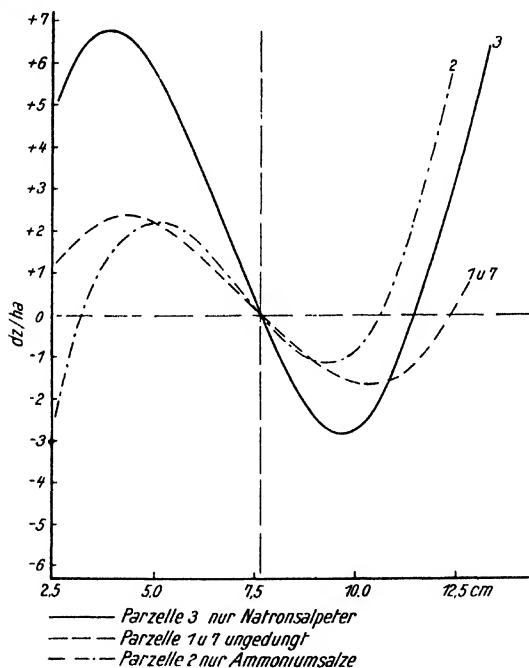


Abb. 6.

Die Kurven zeigen die linearen und die einfachen nicht linearen (kubischen) Wirkungen der März- und April-Niederschläge auf den Korntrag bei Gerste

Im Vergleich zu den Pflanzen der mit Stallmist und schwefelsaurem Ammoniak behandelten Parzelle ist in den Zeiten mit trockenem Frühjahr das Getreide auf der mit Natronsalpeter gedüngten Parzelle wesentlich

besser gekommen; auch die Mineralsalzdüngungen zeigten eine gute Wirkung. Dagegen stand in den Jahren mit feuchtem März und April die schwefelsaure Ammoniak-Parzelle bedeutend besser¹⁾. Stallmistdüngung zeigte eine Veränderung im entgegengesetzten Sinn; sie verschlechterte sich deutlich in den Zeiten mit feuchtem Frühjahr. Man hat den Eindruck, als ob ihre Fähigkeit, die Bodenfeuchtigkeit zu erhalten, sich in feuchter Vegetationszeit auf das Wachstum der Pflanzen ungünstig auswirkt. Andererseits konnte man im trockenen Frühjahr keinen besonders günstigen Einfluß der Stallmistdüngung feststellen.

Diese Folgerung ist in der nachstehenden Tabelle zum Ausdruck gebracht, in der 30 Jahre, 1877 bis 1906, nach der im April festgestellten Niederschlagsmenge geordnet sind.

Die Zahlen dienen nur zur Erläuterung, einen anderen Wert haben sie nicht; die Methode ist nicht durchgearbeitet und für Versuche gänzlich ungeeignet. — Es muß noch hinzugefügt werden, daß die Düngung mit Natronsalpeter Ende April oder Anfang Mai, d. h. nach dem fraglichen Zeitpunkt, vorgenommen wird.

Tabelle 27

April-Niederschläge in Millimeter	Anzahl der Jahre	Die Steigerung des Gesamtertrages in Doppelzentner je Hektar im Vergleich zu der ohne Stickstoff gedüngten Parzelle 4			Erhöhung der Erträge durch Natronsalpeter verglichen mit schwefelsaurem Ammoniak
		Natronsalpeter Parzelle 6	Schwefelsaures Ammoniak Parzelle 5	Stallmistdüngung Parzelle 11 b	
Unter 25 mm	8	27,0	1,7	28,5	22,3
Unter 25—50 mm	13	32,1	10,9	27,8	21,2
Über 50 mm	9	30,6	21,2	18,7	9,4

Niederschläge im Frühsommer.

Ein anderer Zeitraum mit deutlichem Einfluß der Niederschläge auf die Gerstenernte ist der dritte Monat der Vegetation, d. h. 60 bis 90 Tage nach der Aussaat. In diese Periode (die durchschnittlich vom 24. Mai bis 22. Juni dauert) fällt das schnellste Wachstum der Pflanze und damit auch die höchsten Anforderungen an die Bodenfeuchtigkeit; anscheinend wird die gesamte durchschnittliche Regenmenge (55,8 mm) gebraucht. Für die meisten Parzellen genügt diese Menge, und weitere Niederschläge sind schädlich; aber die beiden Parzellen, die mit der doppelten Menge Natronsalpeter und schwefelsaurem Ammoniak gedüngt waren und das stärkste Wachstum bewirken, benötigen größere Niederschlagsmengen; sie brauchen weitere 12,5 mm. Ein Mangel an Regen scheint schädlich

¹⁾ Die Regressions-Gleichungen drücken die Beziehungen zwischen Niederschlägen und der durch Stickstoffdüngung hervorgerufenen Ertragssteigerung aus und deuten darauf hin, daß die Natronsalpeter-Parzellen durch die April-Regen weniger stark geschädigt werden als die Parzellen mit schwefelsaurem Ammoniak. Auf jeden Fall ist der April-Regen für einen geringen Anteil der Jahres-Abweichungen verantwortlich.

zu sein, so daß die durchschnittliche Niederschlagsmenge doch wohl das Optimum darstellt.

Die durch Mangel oder Überschuß an Niederschlägen in dieser Zeit eingetretenen Verluste am Kornertrag, wie sie aus Fräulein Websters durch kubische und quadratische Größen dargestellte Kurven abgelesen worden sind, werden in Tabelle 28 zum Ausdruck gebracht und betragen in Doppelzentner je Hektar:

Tabelle 28.

	Par- zelle	Mangel an Regen		Überschuß an Regen	
		50 mm	25 mm	25 mm	50 mm
Ungedüngt	1	2,62	0,58	1,16	4,08
Nur Mineralsalze	4	3,20	0,58	0,58	3,49
Volldüngung (Natronsalpeter)	6	5,82	1,75	0,58	3,49
	9	6,70	2,33	—	1,46
Volldüngung (schwefelsaures Ammoniak)	5	1,16	—	1,16	4,08
	8	5,82	1,75	—	2,33
Stallmist-Düngung	11 b	3,49	0,58	1,46	4,66

Für Jahre mit einem trockenen Juni hat sich Stallmistdüngung günstiger erwiesen als künstliche Düngung mit Natronsalpeter, aber in einem feuchten Juni hat man mit künstlichen Düngemitteln bessere Erfahrungen gemacht, besonders bei Anwendung stärkerer Stickstoffdüngungen.

Bei Verabreichung höherer Gaben läßt sich zwischen Natronsalpeter und schwefelsaurem Ammoniak ein Unterschied nicht feststellen, obgleich geringere Gaben von schwefelsaurem Ammoniak im trockenen Juni eine höhere Wirksamkeit zu zeigen schienen, eine Eigenschaft, die im Gegensatz steht zu ihrem Verhalten bei trockenem März und April. Im allgemeinen hat man den Eindruck, als ob Natronsalpeter für trockene Sommer geeigneter ist; eine Erläuterung ist in Tabelle 29 gegeben.

Man kann das Ergebnis einerseits ebenso einer absoluten Überlegenheit des Natronsalpeters in Jahren mit trockenem März und April zuschreiben wie es auch durch Unterschiede in der Löslichkeit verursacht sein kann, und andererseits läßt sich das Ergebnis ebenso auf die Unterlegenheit des schwefelsauren Ammoniaks in trockenen Jahren zurückführen wie es auch durch die in trockenen Jahren wohlbekannte Verstärkung der Säurewirkung bedingt sein kann. Der zuletzt genannte Grund kann ausschlaggebend sein, da Mineralsalze allein, ohne Natronsalpeter, auch in trockenen Jahren höhere Erträge gaben als Düngungen mit schwefelsaurem Ammoniak.

Niederschläge im Spätsommer.

Die statistischen Untersuchungen deuten hin auf einen schädlichen Einfluß der Spätsommerniederschläge auf alle, besonders aber auf die mit Natronsalpeter behandelten Parzellen.

Tabelle 29.

Die nach dem Unterschied im Gesamtertrag (Doppelzentner je Hektar) auf vergleichbaren Parzellen in einem Zeitraum von 20 Jahren (1877 bis 1896) festgestellte Überlegenheit von Natronsalpeter über schwefelsaures Ammoniak.

	Gerste			Weizen		
	Trockene Jahre ¹⁾	Feuchte Jahre ¹⁾	Mittlerer Ertrag für alle Jahre	Trockene Jahre	Feuchte Jahre	Mittlerer Ertrag für alle Jahre
Durchschnittliche Niederschlagsmenge in Millimeter, März bis August	2,45	3,45	2,95	2,45	3,45	2,95
Unterschied zwischen den Parzellen						
3 bis 2	6,22	2,26	4,24	2,92	— 2,12 ²⁾	0,40
6 bis 5	11,11	4,68	7,90	5,20	0,74	2,97
9 bis 8	14,02	3,14	8,58	3,50	— 5,91 ²⁾	— 1,21 ²⁾

Die Beziehungen zwischen Ernte, Aussaatzeit und Niederschlägen während der Aussaatzeit.

Während einer dreißigjährigen Versuchszeit lag der Termin für die Aussaat der Gerste zwischen dem 26. Februar (1898) und dem 17. April (1901). Ein bestimmter Einfluß auf den Ertrag läßt sich nicht nachweisen. Es sind auch keine Anzeichen dafür vorhanden, daß frühere Aussaatzeiten die Erträge günstiger beeinflussen als späte Aussaattermine.

Man hat oft gemeint, daß die Hauptsache für gute Erträge eine frühe Aussaat sei. Viele Versuche haben bewiesen, daß bei Gerste, wenn sie nicht zu dem von der praktischen Erfahrung als günstigster anerkannten Aussaattermin sondern früher ausgelegt wird, im Frühstadium eine schwere Wachstumshemmung eintreten kann, von der sich die Pflanze niemals vollständig erholt. Ein Verschieben der Aussaat bis über den für die Pflanzen schädlichen Zeitpunkt hinaus beeinträchtigt die Ernte insofern, als die Pflanze dann zur vollständigen Entwicklung nicht mehr genügend Zeit hat.

Das Ergebnis bringt nicht viel Neues, abgesehen davon, daß die Versuchsleiter immer günstige Aussaattermine gewählt hatten. Hingewiesen wurde bereits auf die Tatsache, daß Abweichungen in den Niederschlägen während der Aussaatzeit keinen merklichen Einfluß auf die Ernte ausüben. Dies mag auch im Zusammenhang stehen mit der sorgfältigen Auswahl der Aussaat auf einen Zeitpunkt, für den die Bedingungen — nach der Ansicht des erfahrenen Praktikers — am günstigsten sind.

Die Wirksamkeit von Natronsalpeter und Stallmist.

Auf den mit Natronsalpeter behandelten Parzellen zeigen die Steigerungen der Gerstenerträge geringere Schwankungen von einem Jahr

¹⁾ Zehn Jahre mit einer überdurchschnittlichen, zehn Jahre mit einer unterdurchschnittlichen Regenmenge.

²⁾ Schwefelsaures Ammoniak ist hier Natronsalpeter überlegen.

zum anderen als die absoluten Werte der Erträge auf den ohne Stickstoff behandelten Parzellen, ein weiterer Beweis für die auf Seite 197 gemachten Ausführungen, daß die prozentischen Abweichungen in den Erträgen auf den Parzellen mit Volldüngung geringer sind als auf denen ohne Stickstoff. Hinsichtlich der Niederschläge hat man den Eindruck, als ob die tatsächliche, durch Natronsalpeter bewirkte Zunahme in feuchten Jahren nicht größer ist als in trockenen Jahren: aber wenn man sie als prozentuale Zunahme auswertet, ist sie doch in feuchten Jahren größer, weil die absoluten Erträge auf den Parzellen ohne Stickstoff dann abnehmen. Es fällt zunächst auf, daß man in den Jahren mit trockenem Frühjahr auf diesem leichten Boden mit Stallmistdüngung nicht bessere Erfahrungen gemacht hat als mit künstlichen Düngemitteln; man muß aber andererseits bedenken, daß die Niederschläge im Frühjahr fast immer zu stark sind und daher der Vorteil des Stallmistes, die Feuchtigkeit zu halten, nicht ausgenutzt werden kann. Nur in Jahren mit trockenen Sommermonaten hat sich die Düngung mit Stallmist günstiger erwiesen als die mit künstlichen Düngemitteln.

Die durchschnittliche Niederschlagsmenge, die für Woburn 608 mm jährlich beträgt, erscheint für einen leichten Boden sicherlich gering, aber die allgemeine Erfahrung dort hat gelehrt, daß diese Menge genügt. In den sehr trockenen Sommern der Jahre 1933 (445 mm Niederschläge) und 1934 (495 mm Niederschläge) zeigten die unter den üblichen Bedingungen angebauten Pflanzen ein überraschend gutes Wachstum. Dieses läßt sich vielleicht mit einer gewissen Besonderheit des Bodens in Woburn erklären oder vielleicht vertragen Gerste und Weizen auch auf anderen leichten Böden trockene Sommer doch besser, als manchmal angenommen wird. Das Stroh kann kurz ausfallen und die Pflanzen mögen einen verkümmerten Eindruck machen, aber schließlich fällt die Ernte doch oft weit besser aus, als man erwartet hatte. Wahrscheinlich liegt der schlechte Ruf der leichten Böden in trockenen Sommern eher an den geringen Erträgen bei Wurzelfrüchten und bei Weideflächen als in einer ersten Schädigung der Getreidepflanzen.

Ein Vergleich zwischen den Wirkungen der Niederschläge in Rothamsted und in Woburn.

Die Wirkungen der Niederschläge in Rothamsted und in Woburn lassen sich nicht genau miteinander vergleichen, weil der Unterschied in der Versuchsdauer sehr groß ist und auch die nicht in Woburn, sondern allein in Rothamsted angewendete „Polynomial“-Methode dazu beiträgt, daß nur auf lange Sicht geradlinige Wirkungen festgestellt worden sind. Es scheint, als ob diese Einflüsse der Niederschläge an beiden Orten bei den drei Parzellen, die sowohl in Woburn als auch in Rothamsted ¹⁾ mit Ergebnissen einer 50jährigen Versuchszeit zur Verfügung stehen, im

¹⁾ Die Ergebnisse einer nur 30jährigen Versuchszeit bringen diese verschiedenen Punkte nicht so deutlich zum Ausdruck.

wesentlichen denselben Charakter zeigen. Für beide ist die durchschnittliche Niederschlagsmenge¹⁾ höher, als es dem Bedürfnis der Pflanzen — auf jeden Fall während des größten Teils der Vegetationszeit — entspricht. Weitere Niederschläge, wann sie auch auftreten, sind fast immer schädlich, besonders aber im Frühjahr: allerdings ist in diesem Fall dann der schädliche Einfluß des Regens in Rothamsted nicht so stark wie in Woburn. Für die ungedüngten Parzellen stimmen die Kurven weitgehend überein, abgesehen, daß Woburn unter der durch Frühjahrsniederschläge verursachten Verschlechterung mehr zu leiden hat als Rothamsted. Folgende Werte ergeben sich für eine Berechnung in Doppelzentner je Hektar:

Wirkung von 25 mm weiteren Niederschlagsmengen.

	Woburn	Rothamsted
Durchschnittliche allgemeine Ertragsdrückung	0,3	0,23
Ertragsdrückung durch Frühjahrsniederschläge	1,3	0,4

Für die anderen Parzellen stimmen die Werte weniger überein, obgleich man auch hier nicht grundlegende Unterschiede feststellen kann. Auf den nur mit Mineralsalzen gedüngten Parzellen ist die durch Frühjahrsniederschläge hervorgerufene Ertragsdrückung an beiden Orten gleich, d. h. sie beträgt 0,5 dz je Hektar; aber sie setzt in Rothamsted später, d. h. im März bis April, ein, während sie sich in Woburn bereits im Februar bemerkbar macht.

Auf Parzellen mit Volldüngung sind sowohl in Rothamsted als in Woburn die jährlichen Abweichungen im Verhältnis zum Ertrage geringer, als dies auf Parzellen nur mit Mineralsalzgaben oder auf ungedüngten Parzellen der Fall ist, und die durch die stickstoffhaltigen Düngemittel bedingten prozentualen Steigerungen der Erträge (allerdings nicht die tatsächlichen Steigerungen) sind größer in Jahren mit feuchtem Frühjahr als bei einer im Frühjahr auftretenden Trockenheit²⁾.

Ähnliche Ergebnisse erhält man auch bei den Parzellen, die nur Stickstoff (Natronsalpeter in Woburn, schwefelsaures Ammoniak in Rothamsted) erhalten haben. Die durch die Frühjahrsniederschläge hervorgerufene Ertragsdrückung wirkt sich auf beiden Stationen in gleichem Maße aus; allerdings kann man in Rothamsted größere Schwankungen feststellen als in Woburn. Bei Stallmistparzellen stimmen die Ergebnisse im allgemeinen überein; doch machen sich in Woburn die Einflüsse der Niederschläge noch mehr bemerkbar als in Rothamsted. Die Ertragsschwankungen betragen in Doppelzentner je Hektar:

¹⁾ 730 mm in Rothamsted, 605 in Woburn.

²⁾ Die Ergebnisse für die Natronsalpeterparzellen in Rothamsted sind noch nicht ausgearbeitet worden, so daß an ihre Stelle die Ergebnisse der mit schwefelsaurem Ammoniak behandelten Parzellen eingesetzt sind. Diese stimmen im großen und ganzen mit den Natronsalpeter-Parzellen überein.

Wirkung von 25 mm weiteren Niederschlagsmengen.

	Woburn (Versuchszeit von 30 Jahren)	Rothamsted (Versuchszeit von 70 Jahren)
Oktober ¹⁾	0,9	+ 0,5
Februar	— 1,9	— 0,8
Juni bis Juli	— 0,5	+ 0,3

Die durch die weiteren Niederschlagsmengen im Frühjahr hervorgerufene Ertragsdrückung läßt sich hauptsächlich damit erklären, daß das Nitrat aus dem Boden ausgelaugt wird: der Verlust kann durch Düngung mit Natronsalpeter und schwefelsaurem Ammoniak wieder ausgeglichen werden. Die Tatsache, daß zum Zustandekommen dieses Ausgleichs ein großer Stickstoff-Überschuß notwendig ist, deutet darauf hin, daß außer dem Verlust an Nitrat noch irgend ein anderer Faktor mit schädigender Wirkung vorhanden sein muß.

Die durch weitere Niederschläge im Juli bedingte Verschlechterung läßt sich nicht durch Düngung mit Natronsalpeter aufheben, ein Beweis, daß die Ertragsdrückung durch andere Faktoren, wie Lagern des Getreides, störende Einwirkungen während des Reifungsprozesses u. ä., verursacht wird.

Fehlen der Wirkung, die dann eintritt, wenn die Erträge sehr stark gesunken sind, zeigt, daß Änderungen in der Nitratzufuhr nicht die einzigen Faktoren sind, die an der Ertragsdrückung beteiligt sind; dies wird auch deutlich durch die Tatsache, daß die Steigerung der Erträge je Kilogramm zugeführten Stickstoffs dann nachläßt, wenn sich die Ertragsdrückung deutlicher bemerkbar macht.

Ein Vergleich zwischen Rothamsted und Woburn hinsichtlich der Verschlechterung der Erträge.

Ein Vergleich zwischen den Ergebnissen von Rothamsted und von Woburn läßt sich schwer durchführen, weil die Dauer der Versuchszeiten ganz verschieden ist: Die Versuchsreihen in Rothamsted erstrecken sich über 70 Jahre, die in Woburn dagegen nur über 30 und 50 Jahre. Es ist klar, daß die bei den ungedüngten, nur mit Mineralsalzen und nur mit Stickstoff gedüngten Parzellen beobachtete Ertragsdrückung sich in Woburn stärker bemerkbar macht als in Rothamsted: Die sowohl an Stickstoff als auch an Mineralsalzen vorhandenen Reserven scheinen auf dem leichten Boden in Woburn schneller verbraucht zu sein als auf dem schwereren in Rothamsted. Auf den mit Stallmist gedüngten Parzellen kann man an beiden Stationen kein Nachlassen in der Wirkung bemerken; auch auf den Parzellen mit Volldüngung läßt sich eine Ertragsdrückung kaum feststellen: Ist sie aber vorhanden, dann scheint sie in Woburn etwas stärker zu sein als in Rothamsted.

¹⁾ Vor der Aussaat im Winter.

Der Unterschied zwischen den beiden Stationen zeigt sich am deutlichsten bei den mit schwefelsaurem Ammoniak gedüngten Parzellen. Eine Versäuerung des Bodens hat man bis jetzt in Rothamsted nicht beobachten können, folglich setzte hier nicht dieses schroffe Abfallen der Erträge ein, wie es in Woburn stattfand.

II. Die Weizen-Versuche.

Die Weizenversuche wurden genau nach denselben Richtlinien durchgeführt wie die Gerstenversuche; Anordnung und Umfang der Versuche sowie Düngung waren bei beiden Getreidearten die gleichen. Während der ersten 15 Jahre, besonders aber in den Jahren 1882 bis 1886, waren die Erträge hoch; sie bewegten sich zwischen 11,4 dz je Hektar auf der ungedüngten Parzelle und 26,2 dz je Hektar auf der Parzelle mit künstlicher Volldüngung. Später nahmen die Ernten auf allen Parzellen ab, allerdings ging das Abfallen der Erträge auf der mit Stallmist gedüngten Parzelle durchweg am langsamsten vor sich.

In normalen englischen landwirtschaftlichen Betrieben kann man bei Weizen im allgemeinen einen Gesamtertrag feststellen, der den der Gerste um 50 % übersteigt. In Woburn liegen die Bedingungen für Weizen jedoch weniger günstig, und im allgemeinen war hier der Gesamtertrag für Weizen und Gerste der gleiche, obgleich Weizen im Herbst und Gerste erst im Frühjahr ausgesät wird. Überdies ist hier die Trennung des Pflanzenmaterials in Korn und Stroh nicht so genau durchgeführt worden wie bei der Gerste: Zwischen 35 und 40 % der gesamten Pflanzensubstanz rechnet zum Korn gegenüber 45 % bei Gerste¹⁾. Folglich liegen die Korn-erträge bei Weizen niedriger als bei Gerste, selbst wenn der Gesamtertrag fast gleich ist. Demgegenüber trat in dem Verhältnis, Korn-ertrag zu Gesamtertrag fast keine Abweichung auf, und erst in den letzten fünf Versuchsjahren ließ sich ein starker Abfall feststellen; hier senkte sich das Verhältnis auf 29 %. Auf den Parzellen ohne Stickstoff wird der Kornanteil in Jahren mit guter Ernte gewöhnlich höher als bei den schlechten Ernten; dagegen neigen die mit Stickstoff gedüngten Parzellen unter den gleichen Bedingungen mehr zu einer Abnahme des Korn-ertrages.

Wie bei Gerste wird auch bei Weizen das Verhältnis von Korn-ertrag zu Strohertrag durch die Düngungsart wenig beeinflusst. Eine Ausnahme hiervon bilden nur die Parzellen mit Natronsalpeterdüngung in doppelter Menge, die relativ mehr Stroh bringen und auf diese Weise gegenüber den ohne Stickstoff behandelten Parzellen ein niedrigeres Verhältnis haben, während bei den mit schwefelsaurem Ammoniak gedüngten Parzellen der Strohertrag geringer ist und sich damit auch gegenüber der Natronsalpeter-Parzelle das Verhältnis Korn-ertrag zu Gesamtertrag erhöht.

Es fällt auf, daß man bei den meisten Düngungsarten in Woburn fast den gleichen Gesamtertrag für Weizen und Gerste feststellen kann.

¹⁾ In Kanada, wo der Weizen im Frühjahr ausgelegt wird, ist das Verhältnis von Korn-ertrag zu Gesamtertrag im allgemeinen höher als in England; es beträgt ungefähr 45 %, derselbe Wert, der in Woburn für Gerste ermittelt wurde.

In einzelnen Jahren sind zwar Unterschiede vorhanden, sie gleichen sich aber bei der Feststellung des Mittelwertes wieder aus.

Sowohl bei Weizen als auch bei Gerste geht fast während der ganzen, sich über 50 Jahre erstreckenden Versuchszeit die Abnahme der Gesamterträge beinahe in demselben Verhältnis vor sich; ist ein Unterschied wirklich festzustellen, wirkt er sich gewöhnlich zu Gunsten des Weizens aus. Die Erträge sind bei Weizen gewöhnlich stabiler als bei Gerste, eine Feststellung, die man besonders auf den sauren Parzellen 2, 5 und 8 machen kann, da Weizen auf saurem Boden besser gedeiht als Gerste. In Tabelle 31 sind die durchschnittlichen Erträge jedesmal für einen Zeitraum von fünf Jahren zusammengefaßt.

1. Die ungedüngten Parzellen.

Die beiden ungedüngten Parzellen brachten sehr ähnliche Erträge; bei Weizenparzellen war der Unterschied geringer als bei Gerste. Während der ersten 15 Jahre waren die Ernten gut, durchschnittlich 31,8 dz je Hektar Gesamtertrag; dann setzte eine Abnahme der Erträge ein und in den nächsten 15 Jahren betrug der durchschnittliche Wert nur 19,3 dz je Hektar, in dem dritten Zeitraum von 15 Jahren sanken die Erträge noch weiter bis auf 17,4 und fielen schließlich während der letzten fünf Jahre schroff ab.

In dem Verhältnis von Kornertrag zu Gesamtertrag konnte man im Gegensatz zu den ungedüngten Gerstenparzellen bei Weizen keine beständige Abnahme beobachten: Das Verhältnis ist hier für die ersten sowohl wie auch für die letzten 15 Jahre das gleiche (31 %); während der dazwischen liegenden 30 Jahre schwankt das Verhältnis nur wenig um 38 %. In demselben Maße wie das Korngewicht verändert sich auch das Gesamtgewicht, aber das Abfallen geschieht nicht ganz so stark.

2. Mineralsalzdüngungen ohne Stickstoff.

Die nur mit Mineralsalzen gedüngten Parzellen ergaben fast dieselben Gesamterträge und Kornerträge wie die ungedüngten Parzellen, eine Tatsache, die man auch bei Gerste feststellen konnte.

Tabelle 30 gibt die nach Doppelzentner je Hektar berechneten Werte an.

Tabelle 30.

Düngung	Gesamtertrag			Kornertrag		
	Die ersten 15 Jahre	Die zweiten 15 Jahre	Die letzten 15 Jahre	Die ersten 15 Jahre	Die zweiten 15 Jahre	Die letzten 15 Jahre
Ungedüngt	31,8	19,3	15,6	10,6	7,2	5,9
Mineralsalzdüngung . .	32,7	17,8	16,6	10,8	6,3	5,8

3. Mineralsalzdüngungen.

Von Stickstoff abgesehen, ist die Wirkung der Mineralsalze sehr gering, geringer als bei Gerste, ein Beweis dafür, daß Weizenpflanzen

mehr als Gerstenpflanzen befähigt sind, aus dem Boden die für sie zur Entwicklung notwendigen Mengen an Kali und Phosphorsäure aufzunehmen. Bei gleichzeitigen Stickstoffgaben steigern die Mineralsalze die Erträge, eine ausgesprochene Wechselwirkung, die darin begründet liegt, daß die Wirkung der Volldüngung weit stärker ist als die Summe, die sich aus der Wirkung des Stickstoffs für sich und der Wirkung der Mineralsalze allein ergibt. Bei Weizen läßt die Wirksamkeit der Mineralsalze im Vergleich zu der ersten Versuchszeit in den späteren Jahren nach zum Unterschied von Gerste (S. 168), bei der die Mineralsalzdüngungen in den letzten 20 Jahren genau so wirksam waren wie in den ersten zehn und bei welcher sich sogar in der Zwischenzeit eine Erhöhung der Wirksamkeit feststellen ließ. Obgleich Anzeichen dafür vorhanden sind, daß Phosphorsäure wirksamer ist, läßt sich doch bei Weizen ebensowenig wie bei Gerste mit Sicherheit feststellen, ob Kali oder Phosphorsäure eine stärkere Wirkung zuzuschreiben ist (Tabelle 31).

Tabelle 31.
Durchschnittlicher Gesamtertrag bei Weizen in Doppelzentner je Hektar.

Parzelle	1877 bis 1881	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
1 und 7 . . .	32,2	34,0	29,3	20,4	18,0	19,5	19,6	16,1	16,3	10,1
4 a	35,0	33,7	29,5	19,7	16,7	16,9	18,2	15,2	20,1	12,9
2 a	42,9	51,7	44,0	37,8	25,2	6,5	3,1	2,4	2,6	2,1
3 a	42,6	50,7	48,5	36,2	36,8	33,0	44,1	27,5	31,4	28,3
3 b	—	—	—	—	—	—	36,5	22,6	28,4	21,5
5 a	56,7	64,4	57,6	46,7	45,5	39,0	31,8	24,4	21,1	16,6
6	57,9	69,9	66,7	42,8	46,2	44,0	40,4	27,2	34,9	23,8
8 a und b										
Mit Stickstoff	73,8	82,6	74,0	52,6	49,9	32,9	14,0	7,6	10,8	5,4
Ohne „	—	36,0	41,8	46,5	36,8	21,6	11,0	6,6	9,2	2,6
9 a und b										
Mit Stickstoff	74,2	85,5	74,2	43,8	56,8	58,6	41,7	29,3	36,3	24,9
Ohne „	—	32,7	30,2	27,1	20,8	25,0	22,2	17,1	19,1	13,3
10 a	—	36,6	34,5 ¹⁾	23,9	23,4	22,5	38,9	25,7	26,6	27,0
10 b	37,9	45,7	50,4 ²⁾	44,2	49,9	51,9	37,8	30,3	28,7	17,7
11 a	—	37,8	35,4	27,4	23,9	27,2	36,3	23,9	27,1	26,0
11 b	45,9	58,4	57,1	43,7	45,7	49,6	45,4	35,8	44,9	26,8

4. Stickstoffhaltige Düngemittel.

Stickstoffhaltige Düngemittel erhöhen die Erträge; die Zunahme hängt von der Menge des zugeführten Stickstoffs ab und wird noch gesteigert durch gleichzeitige Düngung mit Mineralsalzen. In Tabelle 32 sind die Erträge bei Natronsalpeterdüngung in Doppelzentner je Hektar berechnet.

Tabelle 34 und 36 zeigen, daß die durch einfache Stickstoffgaben (Parzelle 6) hervorgerufenen Steigerungen des Gesamtertrages während der ersten 30 Jahre bei beiden Getreidearten weitgehend übereinstimmen,

¹⁾ 1889 Rapskuchen wie 10 b.

²⁾ 1887 Stallmistdüngung, 1888 ungedüngt, 1889 Rapskuchen (0,46 dz Stickstoff), 1890 bis 1906 Rapskuchen (0,92 dz Stickstoff).

Tabelle 31a.
Durchschnittlicher Kornertrag bei Weizen in Doppelzentner je Hektar.

Parzelle	1877 bis 1881	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
1 und 7	10,4	11,5	9,6	7,7	6,7	7,2	7,6	6,3	6,5	3,1
4 a	10,8	11,9	9,6	7,1	5,8	5,9	6,7	5,5	7,4	3,7
2 a	13,9	18,6	15,9	15,6	10,5	2,1	0,7	0,2	0,6	0,2
3 a	13,4	16,7	16,3	12,7	12,7	11,1	15,6	9,1	12,0	8,4
3 b	—	—	—	—	—	—	13,2	8,2	10,7	7,1
5 a	17,8	23,1	20,0	18,7	19,0	15,9	12,6	9,6	8,6	4,1
6	18,1	24,2	22,7	16,2	17,6	15,8	11,5	10,0	14,2	7,1
8 a	22,5	28,4	25,3	21,7	20,7	12,7	4,7	2,4	1,0	1,3
8 b	—	13,5	15,0	17,7	14,8	7,6	3,8	2,4	3,6	0,7
9 a	21,7	26,7	22,6	16,0	20,9	20,6	15,8	9,8	14,4	6,3
9 b	—	11,2	10,5	10,7	7,8	9,1	8,2	6,1	8,0	3,5
10 a	—	12,4	12,3	9,1	9,4	8,7	14,4	10,0	10,7	7,9
10 b	12,0	15,4	17,8	16,7	19,1	18,6	14,7	12,0	11,3	5,8
11 a	—	12,8	13,0	10,9	9,8	10,4	13,1	8,6	11,0	6,9
11 b	11,9	20,2	20,5	16,1	17,1	15,4	15,1	12,3	17,1	6,9

aber später, besonders in den Jahren von 1907 bis 1911 und 1917 bis 1921, waren die Erträge bei Weizen besser als bei Gerste. In den Jahren 1909, 1910 und 1911 war die Gerstensorte „Goldthorpe“ angebaut worden, die sich für die Verhältnisse in Woburn nicht günstig erwies.

Tabelle 31b.
Das Verhältnis von Kornertrag zu Gesamtertrag in Prozenten bei Weizen.
Mittelwerte.

Parzelle	Erste Periode 1877 bis 1891			Zweite Periode 1892 bis 1906			Letzte Periode 1907 bis 1926			
	1877 bis 1881	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
1	33	33	34	36	36	34	38	38	38	30
7	32	35	33	39	38	39	39	40	41	30
4	31	35	33	36	35	35	37	36	38	27
2 ¹⁾	31	36	36	42	42	32	33	35	40	30
3 a	31	33	33	34	35	33	36	33	39	31
3 b	—	—	—	—	—	—	36	35	38	33
5	31	36	35	41	42	41	41	39	41	25
6	30	35	35	39	39	36	36	36	40	31
8 a mit Stickstoff	30	35	34	41	42	39	33	31	36	30
8 b ohne "	—	37	36	39	41	35	34	38	39	34
9 a mit Stickstoff	29	31	30	36	37	35	36	32	40	26
9 b ohne "	—	34	34 ²⁾	40	38	37	37	36	42	26
10 a	—	35	35 ³⁾	40	40	38	37	38	40	29
10 b	33	34	35	39	39	36	39	40	40	33
11 a	—	34	36	40	41	38	36	35	40	27
11 b	32	35	36	38	37	34	35	34	38	26

¹⁾ Die Jahre, in welchen Korn- und Strohmenge nicht eingetragen war, sind bei diesen durchschnittlichen Berechnungen unberücksichtigt geblieben.

²⁾ 1889 Rapskuchen wie 10b.

³⁾ 1887 Stallmistdüngung, 1888 ungedüngt, 1889 Rapskuchen (46 kg Stickstoff), 1890 bis 1906 Rapskuchen (92 kg Stickstoff).

Die Steigerung der Erträge der mit doppelter Stickstoffgabe behandelten Parzelle gegenüber der Parzelle ohne Stickstoff (Parzelle 9) war in den Jahren 1892 bis 1896 nicht so stark wie bei Gerste, sonst war sie für beide Getreidearten gleich.

Stallmistdüngung erhöhte die Erträge bei den beiden Getreidearten ziemlich gleichmäßig. Ausnahmen machten hier die Jahre 1887 bis 1891, in welchen Weizen, 1922 bis 1926, in denen Gerste größere Ertragssteigerungen brachte.

Abb. 2b zeigt dann, wie diese erhöhten Erträge im Laufe der Zeit allmählich wieder sinken: in den letzten fünf Jahren liegen die Werte viel dichter zusammen, als dies in den früheren Zeiträumen — selbst bei Berücksichtigung der verschiedenen Düngungen — der Fall war.

Schwefelsaures Ammoniak.

In den ersten 20 Jahren zeigte sich schwefelsaures Ammoniak in demselben Maße wirksam wie Natronsalpeter, aber dann verschlechterten sich die Erträge: das Nachlassen der Erträge trat bei Weizen fünf Jahre

Tabelle 32

Stickstoff- düngung Kilogramm je Hektar	Parzelle	Gesamtertrag Doppelzentner je Hektar			Kornertrag Doppelzentner je Hektar		
		Die ersten 15 Jahre 1877 bis 1891	Die zweiten 15 Jahre	Die letzten 20 Jahre	Die ersten 15 Jahre	Die zweiten 15 Jahre	Die letzten 20 Jahre
0,46	3	47,3	35,3	32,8	15,4	12,2	11,3
0,46	6	64,8	44,3	31,6	21,7	16,5	11,5
0,92	9	78,2	53,0	33,8	23,6	19,1	11,5

später ein als bei Gerste, aber als das Sinken erst einmal eingesetzt hatte, erfolgte es bei Parzelle 2 (nur schwefelsaures Ammoniak) gleichmäßig schroff, bei Parzelle 5 (schwefelsaures Ammoniak und Mineralsalzdüngungen) dagegen nicht in demselben Maße.

Beim Weizen-Kornertrag sind die Werte sowohl für die absoluten Erträge als auch für die Ertragssteigerungen in den ersten Jahren größer als bei Gerste, werden allerdings in der Folgezeit geringer. Andererseits hat eine am Schluß festgestellte Verschlechterung des Ertrages auf den Kornertrag bei Weizen weniger Einfluß als bei Gerste; folglich sinkt auch das Verhältnis Kornertrag zu Gesamtertrag bei Weizen weit weniger als bei Gerste.

Die zweite Stickstoffgabe beeinflusst die Ernte nicht so stark wie die erste und dieses Nachlassen der Wirksamkeit macht sich bei Weizen im wesentlichen im gleichen Maße bemerkbar wie bei Gerste (Tabelle 32).

Tabelle 33.

Nachwirkungen von Natronsalpeter und schwefelsaurem Ammoniak bei Weizen.

	Parzelle	Kornertrag in Doppelzentner je Hektar Natronsalpeter				
		1882	1883	1884	1885	1886
Erträge in den Jahren mit einer Stickstoff- düngung von 92 kg je Hektar	9	23,6	28,4	33,1	25,9	22,4
Erträge in den Jahren ohne Stickstoff		7,6	12,3	14,1	11,8	10,0
Erträge der ständig ohne Stickstoff gebliebenen Parzelle	4	9,4	11,3	14,3	13,9	10,2
Wahrscheinliche Nachwirkung		- 1,8	+ 1,0	- 0,2	- 2,1	- 0,2

	Parzelle	Kornertrag in Doppelzentner je Hektar Natronsalpeter				
		1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906
Erträge in den Jahren mit einer Stickstoff- düngung von 92 kg je Hektar	9	26,7	22,6	16,0	20,8	20,6
Erträge in den Jahren ohne Stickstoff		11,2	10,4	10,7	7,8	9,1
Erträge der ständig ohne Stickstoff gebliebenen Parzelle	4	11,8	9,5	7,0	5,8	5,9
Wahrscheinliche Nachwirkung		- 0,6	+ 0,9	+ 3,7	+ 2,0	- 3,2

Zweite Periode. Halbe Düngung.

	Parzelle	Kornertrag in Doppelzentner je Hektar Natronsalpeter			
		1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926
Erträge in den Jahren mit einer Stickstoff- düngung von 46 dz je Hektar	9	15,8	9,8	14,4	6,3
Erträge in den Jahren ohne Stickstoff		8,2	6,4	8,0	3,5
Erträge der ständig ohne Stickstoff gebliebenen Parzelle	1	6,7	5,5	7,4	3,6
Wahrscheinliche Nachwirkung		+ 1,5	+ 0,9	- 0,6	- 0,1

	Parzelle	Kornertrag in Doppelzentner je Hektar Schwefelsaures Ammoniak				
		1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906
Erträge in den Jahren mit einer Stickstoff- düngung von 46 kg je Hektar	2	28,4	25,3	21,7	20,7	12,7
Erträge in den Jahren ohne Stickstoff		13,5	15,0	17,7	14,8	7,6
Erträge der ständig ohne Stickstoff gebliebenen Parzelle	4	11,8	9,6	7,1	5,8	5,9
Wahrscheinliche Nachwirkung		+ 1,7	+ 5,1	+ 10,6	+ 9,0	+ 1,7

Möglicherweise vorhandene Nachwirkung von Natronsalpeter.

Bei Weizen läßt sich gewöhnlich keine solche ausgesprochene Nachwirkung feststellen wie bei Gerste. In manchen Jahren, vor allem in den Jahren 1894 und 1902, konnte man eine deutliche, in den Jahren 1905 und 1908 eine weniger ausgeprägte Wirkung beobachten; aber in den meisten Jahren bestand nur wenig Unterschied zwischen Parzelle 9b, die nur in dem vorhergehenden, nicht in dem laufenden Jahr mit Natronsalpeter gedüngt war, und Parzelle 4, die überhaupt keinen Natronsalpeter erhalten hatte. Betrachtet man die jedesmal fünf Jahre umfassenden Zeiträume, so kann man weder in den ersten zwei noch in den letzten drei Abschnitten Anzeichen einer Nachwirkung beobachten, in den vier dazwischen liegenden Zeiträumen, von 1892 bis 1911, scheint eine Nachwirkung vorhanden zu sein. Ein Vergleich der Tabellen 19 und 33 zeigt einen deutlichen Unterschied zwischen Weizen und Gerste.

Eine Erklärung hierfür kann nicht gegeben werden; es besteht die Möglichkeit, daß die Unterschiede nur auf Bodenungleichmäßigkeiten beruhen.

Nachwirkung von schwefelsaurem Ammoniak.

Eine deutliche Nachwirkung zeigt schwefelsaures Ammoniak. Während der ersten 10 Jahre ist die Wirkung bei Weizen nicht so stark wie bei Gerste; aber dann nahm die Wirkung plötzlich zu und zwar gerade zu derselben Zeit, als ein Nachlassen der Wirkung auf Gerstenparzellen einsetzte. Später wurde die Wirkung bei Weizen schwächer, aber das Sinken geschah nicht so schroff wie bei Gerste. Die nachfolgende Tabelle stellt die Nachwirkungen von schwefelsaurem Ammoniak dar, wie sie sich aus den Ertragssteigerungen gegenüber Parzelle 4 feststellen ließen.

Gesamtertrag in Doppelzentnern je Hektar.

	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906
Weizen	2,3	12,3	26,8	20,1	4,6
Gerste	16,8	13,9	5,7	0,7	— 7,4

Mit der zunehmenden Versäuerung des Bodens um 1889 setzte eine Schädigung der Gerstenbestände ein und von diesem Zeitpunkt an konnte man ein deutliches Sinken der Erträge bemerken. Weizen war gegen Versäuerung des Bodens weniger empfindlich und hielt seine Erträge noch längere Zeit hindurch auf derselben Höhe. In den allerersten Jahren der Bodenversäuerung (1892 bis 1896), also bevor die endgültige zu starke Versäuerung eingetreten war, konnte man eine deutliche Zunahme der Nachwirkungen feststellen. Dieses deutet darauf hin, daß der niedrigste Grad der Versäuerung, d. h. der Grad, der dem der schädigenden Einwirkungen vorausgeht, sicherlich das Wachstum der Pflanzen fördert. Diese Ansicht wird bestärkt durch das fleckige Aussehen des Getreides, das sich im ersten Stadium der Schädigung befindet und einige Pflanzen von

ungewöhnlich gutem, andere von fast verkümmertem Wachstum zeigt. Umgekehrt kann man die Wachstumszunahme auch damit in Zusammenhang bringen, daß eine der zu dieser Zeit in Woburn sehr oft vorkommenden parasitären Krankheiten des Weizens „Schwarzbeinigkeit“ (*Ophiobolus graminis*) durch die Versäuerung stark beeinträchtigt wird; in einem gewissen Stadium der Bodenversäuerung kann die Weizenpflanze noch wachsen, während der Pilz in seiner Entwicklung gehemmt wird. Leider sind keine Angaben über das damalige Auftreten der Krankheit vorhanden, so daß wir diese Annahme nicht nachprüfen können. Ferner besteht die Möglichkeit, daß die Versäuerung des Bodens die Salpeterbildung im Herbst hemmen und damit den während des Winters auftretenden Nitrat-Verlust verringern kann. Es kann auch sein, daß Ammoniak von der Pflanze wirksamer und

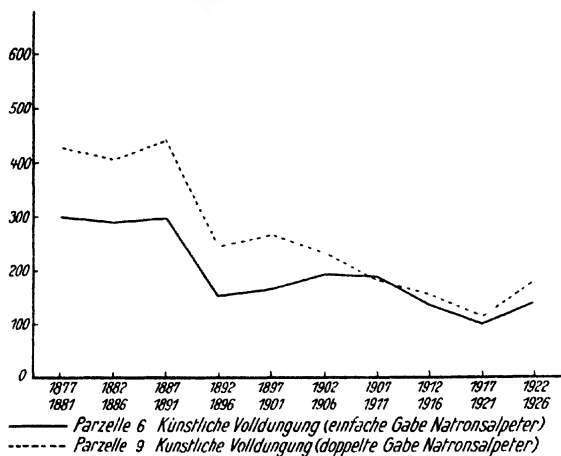


Abb. 7.

Steigerung des Gesamtertrages je Kilogramm zugeführten Stickstoffs
(Steigerung durch Stallmistdüngung = 100)

schneller ausgewertet wird als Nitrat; unter normalen Bedingungen haben sie durch das schnelle Nitrifizieren kaum Gelegenheit, Ammoniak zu erhalten; aber unter diesen außergewöhnlichen Voraussetzungen geht die Salpeterbildung so langsam vor sich, daß dadurch den Pflanzen genügend Möglichkeit zur Ammoniakaufnahme gegeben ist. Welcher Grund auch immer vorliegen mag, jedenfalls hält diese Stimulation der Nachwirkung nur wenige Jahre vor. Später wurden die Weizenernten durch die Bodenversäuerung auch vermindert, allerdings nicht so stark wie bei Gerste.

5. Stallmist.

Stallmistdüngung, die jährlich in einer Menge von ungefähr 200 dz je Hektar gegeben wurde, ergab für die jeweiligen Mittel aus fünf Jahren im wesentlichen bei Weizen und Gerste die gleichen Ergebnisse; allerdings ließen sich in einzelnen Jahren Unterschiede feststellen. In den ersten vier Jahren zeigte Stallmistdüngung bei Weizen kaum einen besonders günstigen Einfluß, denn der Kornertrag war zwar um 2,7 dz höher

als derjenige, der durch eine Volldüngung mit 46 kg Stickstoff als Natronsalpeter erreicht worden war, aber während der gesamten fünf Jahre war die Steigerung des Gesamtertrages bei Weizen nicht so stark wie bei Gerste. In den dann folgenden Zeiträumen stieg die Wirkung des Stallmistes auf ein Maximum, das beständig eingehalten wurde. Anzeichen einer Ertragsdrückung sind hier nicht vorhanden.

Faßt man die durchschnittlichen Ergebnisse von 15 Jahren zusammen, so zeigt es sich, daß der durch Stallmistdüngung erzielte Gesamtertrag für Weizen annähernd der gleiche ist wie für Gerste; in den ersten 15 Jahren ist er etwas höher, in den zweiten 15 Jahren etwas niedriger.

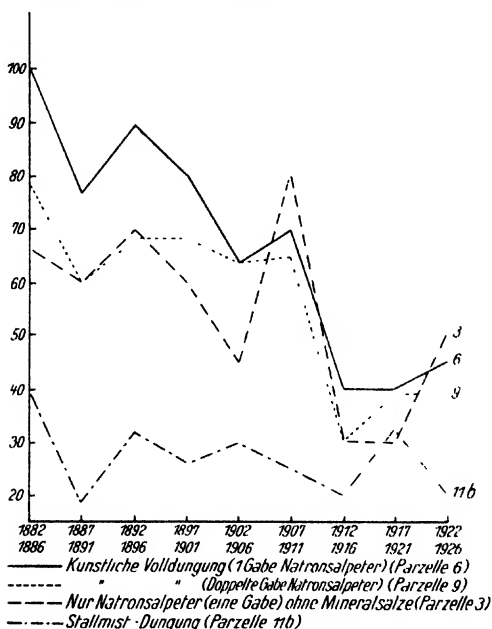


Abb. 8.

Durchschnittliche Stickstoffaufnahme
aus den zugeführten Düngemitteln bei Gerste.

Die durch Stallmistdüngung bewirkten Ertragssteigerungen über die Erträge der ungedüngten oder nur mit Mineralsalzen gedüngten Parzellen sind praktisch dieselben für Weizen und Gerste bis fast zum Abschluß des Versuches, als sich die Ertragssteigerungen bei Weizen eher auf der gleichen Höhe hielten. Angaben darüber bringt Tabelle 36.

Stallmist unterscheidet sich also dadurch von Natronsalpeter und schwefelsaurem Ammoniak, bei denen die Ertragssteigerungen im Laufe der Zeit geringer werden.

Abb. 7 zeigt die Verringerung der Ertragssteigerung je Kilogramm als schwefelsaures Ammoniak und Natronsalpeter zugeführten Stickstoffs verglichen mit der (= 100 eingesetzten) Ertragssteigerung durch Stallmistgaben.

Tabelle 34.

Die durch Düngung mit Stallmist und Natronsalpeter erzielten Ertragssteigerungen, berechnet auf ein Teil zugeführten Stickstoffs.

	Die ersten 15 Jahre 1877 bis 1891		Die zweiten 15 Jahre 1892 bis 1906		Die letzten 20 Jahre 1907 bis 1926	
	Gerste	Weizen	Gerste	Weizen	Gerste	Weizen
Stallmistdüngung Parzelle 11b						
Gesamtertrag	17	17	24	23	25	24
Kornertrag	8	6	10	8	8	11
Natronsalpeter Parzelle 6						
Gesamtertrag	73	70	59	58	42	65
Kornertrag	29	24	21	22	18 ¹⁾	25
	—	—	—	—	15 ²⁾	—
Die Wirksamkeit des Stickstoffs in der Stallmistdüngung						
Gesamtertrag	23	41	41	40	93	54
Kornertrag	28	42	42	36	—	44
(Stickstoff im Natronsalpeter = 100)						

Abb. 8 stellt die prozentische Stickstoffaufnahme aus dem Düngemittel dar, die bei Stallmistgaben annähernd konstant bleibt, bei den anderen Düngemitteln dagegen geringer wird.

Tabelle 35

Die durch Natronsalpeter erzielten Steigerungen im Kornertrag, berechnet in Doppelzentner je Hektar.

Stickstoff im Düngemittel kg/ha	Andere Düngungen	Parzellen	Weizen				Gerste			
			Steigerungen gegenüber den ohne Stickstoff be- handelten Parzellen	Steigerung in Prozenten	Tatsächliche Steigerung je Kilo- gramm zugeführ- ten Stickstoffs	Steigerungen gegenüber den ohne Stickstoff be- handelten Parzellen	Steigerung in Prozenten	Tatsächliche Steigerung je Kilo- gramm zugeführ- ten Stickstoffs ³⁾		
Die ersten 15 Jahre, 1877 bis 1891.										
46	Keine	3	4,8	45	10	8,6	64	19		
46	Mineralsalze .	6	10,9	102	24	13,4	100	29		
92	Mineralsalze .	9	12,9	120	—	16,7	124	—		
Die zweiten 15 Jahre, 1892 bis 1906.										
46	Keine	3	5,0	70	11	7,0	87	15		
46	Mineralsalze .	6	10,2	164	22	11,2	106	24		
92	Mineralsalze .	9	12,9	205	—	15,0	142	—		
Die letzten 20 Jahre, 1907 bis 1926										
23	Keine	3 b	3,9	66	17	1,6	33	7		
46	Keine	3 a	5,4	92	12	2,9	58	6		
23	Mineralsalze .	6	5,7	98	25	4,0	60	18		
46	Mineralsalze .	9	5,7	100	—	6,7	99	15 ⁴⁾		

¹⁾ Parzelle 6, aber mit halber Düngung.

²⁾ Parzelle 9, aber dieselbe Düngung wie vorhergehend auf Parzelle 6.

³⁾ Was Parzelle 3 betrifft, so sind hier die Steigerungen bezogen auf das Mittel der Erträge der Parzellen 1 und 7 und bei den Parzellen 6 und 9 auf die Parzelle 4 nur Mineralsalze).

⁴⁾ Halbe Düngung = 23 kg Stickstoff.

Tabelle 36.
Durch Natronsalpeter hervorgerufene Steigerungen im Gesamtertrag
und Kornertrag bei Weizen.

Stickstoff im Düngemittel kg/ha	Andere Düngungen	Parzellen	Gesamtertrag dz/ha	Steigerung gegen- über den ohne Stick- stoff behandelten Parzellen dz/ha ¹⁾	Kornertrag dz/ha	Steigerung gegen- über den ohne Stick- stoff behandelten Parzellen dz/ha ¹⁾	Steigerung des Gesamt- ertrages je Kilogramm mit dem Düngemittel zugeführten Stickstoffs	Steigerung des Korn- ertrages je Kilogramm mit dem Düngemittel zugeführten Stickstoffs
Die ersten 15 Jahre, 1877 bis 1891.								
46	Keine	3	47,3	15,4	15,4	4,8	34	10
96	(Rothamsted) . .	10 ²⁾	32,1	13,2	12,8	4,7	11	5
46	Mineralsalze . .	6	64,8	32,0	21,7	10,9	70	24
48	(Rothamsted) . .	9	52,4	30,5	18,4	9,2	63	19
92	Mineralsalze . .	9	78,2	45,3	23,6	12,9	49	14
96	(Rothamsted) . .	16	50,4	28,6	17,7	8,4	30	9
124	Stallmistdüngung	11 b	—	—	—	—	17	—
Die zweiten 15 Jahre, 1892 bis 1906.								
46	Keine	3	35,3	16,0	12,2	5,0	35	11
96	(Rothamsted) . .	10	33,1	12,4	13,0	4,2	13	4
46	Mineralsalze . .	6	44,3	26,6	16,5	10,2	58	22
48	(Rothamsted) . .	9	51,7	26,1	19,3	8,6	54	18
92	Mineralsalze . .	9	53,0	35,3	19,1	12,9	38	14
96	(Rothamsted) . .	16	66,2	40,6	23,5	12,8	42	13
124	Stallmistdüngung	—	—	—	—	—	23	—
Die letzten 20 Jahre, 1907 bis 1926								
23	Keine	3 B	—	—	—	—	—	—
46	Mineralsalze . .	3 A	32,8	17,2	11,3	5,1	—	—
23	Mineralsalze . .	6	32,0	15,0	11,5	4,6	—	—
46	Mineralsalze . .	9 A	33,8	17,2	11,6	5,8	—	—
124	Stallmistdüngung	—	—	—	—	—	—	—

Bei Fortführung der Versuche mit der Hälfte Stallmistdüngung kam man zu interessanten Ergebnissen: Während der ersten fünf Jahre war diese Düngung je Einheit zugeführten Stickstoffs nicht annähernd so wirksam wie die doppelte Düngung (dieses Ergebnis stimmte nicht mit dem bei Natronsalpeter und schwefelsaurem Ammoniak überein, aber in den nächsten fünf Jahren zeigte sich eine gleichmäßige Wirkung. Innerhalb der ersten fünf Jahre erwies sich keine der Stallmistdüngungen bei Weizen

¹⁾ Gegenüber den Parzellen 1 und 7 sowie 4.

²⁾ Schwefelsaures Ammoniak.

¹⁾ Nach den ersten fünf Jahren (d. h. nach 1883) lassen sich die doppelten Düngungen nicht genau mit den einfachen vergleichen, weil sie auf der halben Parzelle zur Anwendung kamen, die im Vorjahre keinen Stickstoff erhalten hatte; die einfachen Düngungen hingegen folgten auf einfache des Vorjahres. Auf diese Weise konnte man bei einfachen Düngungen „Nachwirkungen“ feststellen, bei den doppelten aber nicht.

²⁾ Die Werte für die letzten 20 Jahre, 1906 bis 1927, können nicht streng mit den früheren verglichen werden, teils aus dem Grunde, weil der Zeitpunkt der Düngung verlegt worden war, teils aber auch deshalb, weil ein Wechsel in der Düngung selbst eingetreten war: Die Werte je Kilogramm Stickstoff hängen von der Menge des zugeführten Düngemittels ab und brauchen für 22 kg Düngemittel nicht unbedingt die gleichen wie für 44 kg zu sein.

so wirksam wie bei Gerste; aber in dem zweiten Zeitraum waren die Ergebnisse des Gesamtertrages bei den beiden Getreidearten die gleichen, nur konnte man bei Gerste höhere Kornerträge feststellen. Die Ergebnisse sind in Tabelle 35 aufgezeichnet.

Tabelle 37.
Ertragssteigerungen je Einheit zugeführten Stickstoffs
als einfache und doppelte Stallmist- und Natronsalpetergaben.

Zugeführter Stickstoff		Die ersten 5 Jahre 1877 bis 1881		Die zweiten 5 Jahre 1882 bis 1886	
		Weizen	Gerste	Weizen	Gerste
kg					
Stallmistdüngung Parzelle 11 b					
55	Gesamtertrag	5	13	19	19
	Kornertrag	2	5	6	10
Stallmistdüngung Parzelle 11 b					
110	Gesamtertrag	9	14	20	22
	Kornertrag	3	7	7	10
Natronsalpeter Parzelle 6					
46	Gesamtertrag	50	62	79	89
	Kornertrag	16	24	27	36

Nachwirkungen bei Stallmistdüngung.

Die Düngung der Weizenparzellen erfolgte in derselben Weise wie bei Gerste. Parzelle 10 und 11 wurden beide 1882 geteilt. Die eine Hälfte (B) erhielt die gleiche Düngung wie vorher, die andere Hälfte (A) blieb ungedüngt. Es war bereits festgestellt worden, daß in den fünf Jahren von 1877 bis 1881 die mittleren Gesamterträge bei Weizen und Gerste übereinstimmten; Unterschiede traten erst nach dem Fortlassen der Düngung auf. Die Ergebnisse findet man in Tabelle 38.

Tabelle 38.
Gesamterträge der Weizen- und Gerstenparzellen, die 5 Jahre hintereinander
mit Stallmist gedüngt waren, später aber keine Düngung mehr erhielten.

	Jährliche Düngung 1877 bis 1881 Mittel aus 5 Jahren	Anschließend ohne Düngung 1882 bis 1886 Unterschied zwischen den Parzellen A und B				
		1882	1883	1884	1885	1886
Stallmistdüngung 100 Doppelzentner je Hektar.						
Weizen	36,6	7,2	13,2	7,5	13,4	4,0
Gerste	36,8	5,7	10,0	6,8	6,6	9,7
Stallmistdüngung 200 Doppelzentner je Hektar						
Weizen	45,9	17,3	27,8	23,6	20,1	14,0
Gerste	46,7	6,5	18,9	17,5	2,6	4,0

Der durch den Fortfall der Stallmistdüngung auf Parzelle A hervorgerufene Ertragsunterschied war bei Weizen größer als bei Gerste, ein Beweis dafür, daß sich die Nachwirkungen der im Jahr vorher durchgeführten Stallmistdüngung bei Gerste mehr bemerkbar machten als bei Weizen

Tabelle 39.
Einfluß der organischen Düngungen, Stallmist und Rapskuchen,
auf Weizenparzellen.

Düngung	Parzelle	Gesamtertrag in Doppelzentner je Hektar	Steigerung gegenüber der ohne Stickstoff behandelten Parzelle 4	Korntrag in Doppelzentner je Hektar	Steigerung gegenüber der ohne Stickstoff behandelten Parzelle 4	Steigerung je Kilogramm zugeführten Stickstoffs	
						beim Gesamt-ertrag	beim Korntrag
Die ersten 5 Jahre 1877 bis 1881							
Stallmistdüngung 62 kg Stickstoff .	10b	36,6	1,6	12,0	1,2	5	2
Stallmistdüngung 124 kg Stickstoff	11b	45,9	10,9	14,9	4,1	9	3
Natronsalpeter + Mineralsalze 46 kg Stickstoff	6	57,9	22,9	18,1	7,3	50	16
Die zweiten 5 Jahre 1882 bis 1886							
Stallmistdüngung 62 kg Stickstoff .	10b	45,7	12,0	15,4	3,6	19	6
Stallmistdüngung 124 kg Stickstoff	11b	58,4	24,7	20,2	8,3	20	7
Natronsalpeter + Mineralsalze 46 kg Stickstoff	6	69,9	36,2	24,2	12,3	79	27
1890 bis 1899							
Stallmistdüngung 124 kg Stickstoff	11b	49,7	28,0	17,7	10,2	23	8
Rapskuchen 92 kg Stickstoff . . .	10b	57,5	29,9	19,1	11,6	32	12
Natronsalpeter + Mineralsalze 46 kg Stickstoff	6	54,4	29,7	18,8	11,2	65	24
1900 bis 1906							
Stallmistdüngung 124 kg Stickstoff	11b	46,0	30,1	16,6	10,9	24	9
Rapskuchen 92 kg Stickstoff . . .	10b	47,9	32,1	17,7	12,1	35	13
Natronsalpeter + Mineralsalze 46 kg Stickstoff	6	41,2	25,3	15,6	9,9	55	22

Es liegt kein Grund vor, diesen Unterschied den Pflanzen zuschreiben zu wollen. Die Art, wie die Düngung auf beiden Parzellenreihen vorgenommen wurde, zeigte große Abweichungen, die man für das Ergebnis verantwortlich machen dürfte. Bei Gerste wurde die Düngung kurz vor der Aussaat untergepflügt, bei Weizen dagegen kam sie, abgesehen von den beiden ersten Jahren, immer als Kopfdüngung zur Anwendung. Dies beeinflusste den Ertrag nicht, so lange die Düngung jährlich zur Anwendung kam. Man kann annehmen, daß nach dem Fortlassen der Düngegaben das mit dem Boden vermischte Düngematerial stärkere Nachwirkungen besitzt als die oberflächliche und nur dann und wann in den Boden eingezogene Düngung.

Die Nachwirkungen lassen sich berechnen durch einen Vergleich der Gesamterträge der Parzellen 10a und 11a mit denjenigen der ungedüngten Parzellen. Tabelle 40 bringt diese Zahlen.

In den Jahren mit Stallmistdüngung konnte man bei Weizen- und Gerstenparzellen fast dieselben Ertragssteigerungen gegenüber den unge-

düngten Parzellen feststellen. In beiden Fällen erzielte die gesamte Düngergabe mehr als das Doppelte der bei halber Düngung festgestellten Ertragssteigerung, ein Beweis, daß stärkere Düngungen je Einheit zugeführten Stickstoffs wirksamer sind als die schwachen. Auch konnte man weder in dem ersten Zeitraum noch in irgend einem anderen einen großen Unterschied zwischen den Erträgen der ungedüngten Weizen- und Gerstenparzellen feststellen. Nach dem Fortlassen der Düngung ergaben die so lange für Weizen und Gerste gleich starken Nachwirkungen hinsichtlich der Menge sehr große Unterschiede. Die Erträge fielen nicht bis zu dem Stand der ungedüngten Parzellen, sondern blieben während der nächsten 25 Jahre höher; dann wurde der Versuch leider abgebrochen. Mit einer

Tabelle 40.

Steigerung der Gesamterträge auf den Parzellen, die nach anfänglichen Stallmistgaben später ungedüngt geblieben sind (Parzelle 10a und 11a) gegenüber den ständig ungedüngt gewesenen Parzellen 1 und 7.

Parzelle		Jedes Jahr Stallmist- düngung 1877 bis 1881	Keine weiteren Düngegaben				
			1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906
Weizen-Gesamtertrag							
10 a	100 dz/ha	5,7	2,6	5,2	3,5	5,4	3,0
11 a	200 dz/ha	13,6	3,8	6,1	7,0	5,9	7,8
	Verhältnis	2,4	1,5	1,2	2,0	1,1	2,6
	Ertrag, Parzelle 1 und 7 . .	32,2	34,0	29,3	20,4	18,0	19,5
Gerste-Gesamtertrag							
10 a	100 dz/ha	5,5	8,3	8,2	6,4	4,9	6,2
11 a	200 dz/ha	15,1	14,6	17,6	16,4	12,2	13,1
	Verhältnis	2,8	1,8	2,1	2,6	2,5	2,0
	Ertrag, Parzelle 1 und 7 . .	31,4	34,1	24,3	20,3	15,8	16,9

Düngung von 200 dz erreicht man eine stärkere Nachwirkung als mit 100 dz, während der ganzen Versuchsdauer beträgt sie annähernd das Doppelte; bei Gerste ist das Verhältnis 2,2, bei Weizen 1,7. Man kann nicht ohne weiteres glauben, daß fünf jährliche Düngungen mit Stallmist, der jedesmal nur in einer Menge von 100 bzw. 200 dz je Hektar gegeben wird, eine so tiefe Wirkung auf den Boden haben kann. Und doch sind die Ergebnisse ganz gleichmäßig. Es besteht die Möglichkeit einer Zunahme der Bodenfruchtbarkeit, ein Faktor, der nicht nachgeprüft werden kann, dem aber, falls er tatsächlich bestanden hat, die Ergebnisse zugeschrieben werden könnten. Diese Art der Unsicherheit ist enthalten in den alten Methoden des Feldversuchs, sie tritt aber bei den neuen in Rothamsted verwendeten Methoden nicht mehr in Erscheinung.

Dennoch besteht die Möglichkeit, daß Stallmistdüngung eine gewisse Dauerwirkung auf den Boden ausübt. Ein ähnlicher Versuch in Rothamsted brachte ähnliche Ergebnisse. Gerste, die seit 1872 ununterbrochen auf dem von da ab ungedüngten, aber vor dieser Zeit, von 1852 bis 1871,

jährlich mit Stallmist behandelten Hoosfield angebaut war, ergab noch Ernten mit weit höheren Erträgen als auf den angrenzenden ungedüngten Parzellen. Diese Parzellen ähneln zwar hinsichtlich der Behandlung sehr dem Hoosfield, unterscheiden sich aber von diesem dadurch, daß sie auch in denselben 20 Jahren (1852 bis 1871) keine Düngung erhalten haben. Diese Dauerwirkungen des Stallmistes bei Einfelderwirtschaft müssen noch weiter erforscht werden.

Der Versuch gibt keinen Aufschluß darüber, welcher Art die Faktoren sind, die diese Dauerwirkungen des Stallmistes hervorrufen. Bei den Bodenveränderungen mag der Einfluß der organischen Substanz eine wichtige Rolle spielen. Ein gewisser Anteil kann auch den in der Stallmistdüngung vorhandenen Mineralstoffen zugeschrieben werden: Die der Parzelle 10 zugeführten 500 dz Stallmist je Hektar würden 3 bis 4 dz K_2O enthalten, was ungefähr 6 bis 8 dz Kaliumchlorid oder Kalisulfat je Hektar entsprechen würde. Für das Bestehen dieser Möglichkeit spricht ferner die Tatsache, daß die Gerstenenerträge auf Parzelle 10a in den ersten sechs oder sieben Jahren ohne Düngung während der übrigen Versuchszeit mit den Erträgen der Parzelle 4 (nur Mineralsalze) annähernd übereinstimmen. Hingegen bleibt die Weizenernte auf Parzelle 10a noch weit höher als die Ergebnisse der Parzelle 4. Die Steigerung der Erträge gegenüber denjenigen der Parzelle 4 ist aus Tabelle 41 ersichtlich.

Pflichten wir der Ansicht bei, daß die Ergebnisse tatsächlich Nachwirkungen darstellen, so kommen wir damit auf ein schwieriges Problem der Stickstoffzufuhr.

Die Aufstellung der Stickstoffbilanz macht dies sehr deutlich.

	Parzelle 10a Kilogramm je Hektar	Parzelle 11a Kilogramm je Hektar
Der gesamte mit der Stallmistdüngung in 5 Jahren (1877 bis 1881) zugeführte Stickstoff	— 308,3	616,6
Von den Pflanzen entnommener Stickstoff, Steigerung gegenüber der ungedüngten Parzelle:		
Direkte Wirkung in 5 Jahren (1877 bis 1881) . . .	25,8	89,7
Nachwirkung in 25 Jahren (1882 bis 1906) . . .	224,2	470,8
Differenz unbekannter Ursache in Kilogramm	58,3	56,0
Differenz unbekannter Ursache in Prozenten	19	9
Zurückgewonnener Stickstoff in Prozenten	81	91

6. Rapskuchen.

Gegenüber dem Stickstoff im Stallmist ergibt der in Rapskuchen enthaltene Stickstoff sowohl höheren Gesamt- als auch Kornertrag; er zeigt sich auf Weizenparzellen wirksamer als bei Gerste (Tabelle 14 und 42). Dieser Unterschied braucht nicht unbedingt mit den Pflanzen in Zusammenhang zu stehen; es ist eher anzunehmen, daß er sich damit erklären läßt, daß Weizen bereits im Januar gedüngt wird, während die Gerstenparzellen ihre Düngergaben erst im März oder April erhalten.

Tabelle 41.

Die Steigerung der Gesamterträge in Doppelzentnern je Hektar auf den Parzellen, die nach anfänglicher Stallmistgabe später ungedüngt geblieben sind (Parzelle 10a und 11a) gegenüber Parzelle 4, die nur Mineralsalzdüngung erhielt.

Parzellen		Jedes Jahr Stallmist- düngung 1877 bis 1881	Keine weiteren Düngegaben				
			1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906
Weizen							
10 a	100 dz.	1,6	2,9	5,0	4,2	6,7	5,5
11 a	200 dz.	10,9	4,1	5,8	7,8	7,2	10,3
	Verhältnis	6,9	1,4	1,2	1,8	1,0	1,8
	Ertrag Parzelle 4	35,0	33,7	29,5	19,7	16,7	16,9
Gerste							
10 a	100 dz.	7,9	10,7	5,2	2,1	—	2,7
11 a	200 dz.	17,8	17,0	14,6	12,1	7,0	9,5
	Verhältnis	2,3	1,6	2,8			
	Ertrag Parzelle 4	28,9	32,0	27,4	24,7	21,0	20,4

Wie der Stickstoff des Stallmistes, so behält auch derjenige des Rapskuchens seine Wirkung während der zweiten 20 Jahre in der gleichen Höhe wie in den ersten 20 Jahren; eine Abschwächung der Wirkung, die bei dem in Natronsalpeter vorhandenen Stickstoff besteht, läßt sich hier nicht feststellen. Die Ertragssteigerungen je Kilogramm zugeführten

Tabelle 42.

Die Wirkung organischer Düngemittel, Stallmist und Rapskuchen, auf Weizenparzellen.

Düngung	Parzelle	Ertrag				Steigerung gegen- über der Parzelle ohne Stickstoff				Steigerung je Kilo- gramm zugeführten Stickstoffs			
		1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926

Gesamtertrag in Doppelzentner je Hektar.

Stallmistdüngung, 62 kg Stickstoff . .	11b	45,4	35,8	44,9	26,8	27,2	20,6	25,0	13,9	44,0	33,0	40,0	22,0
Rapskuchen, 23 kg Stickstoff . .	10b	37,8	30,3	28,7	17,7	19,6	15,1	8,8	4,8	85,0	66,0	38,0	21,0
Natronsalpeter, 23 kg Stickstoff und Mineralsalze . . .	6	40,4	27,2	34,9	23,8	22,1	12,0	15,0	10,9	96,0	52,0	65,0	48,0

Kornertrag in Doppelzentner je Hektar.

Stallmistdüngung, 62 kg Stickstoff . .	11b	15,4	12,3	17,0	6,9	8,7	6,8	9,6	3,2	14,0	11,0	16,0	5,0
Rapskuchen, 23 kg Stickstoff . .	10b	14,7	12,0	11,2	5,8	8,0	6,5	3,8	2,1	35,0	28,0	16,0	9,0
Natronsalpeter, 23 kg Stickstoff und Mineralsalze . . .	6	14,4	10,0	14,2	7,4	7,8	4,5	6,7	3,7	34,0	20,0	29,0	16,0

Stickstoffs gegenüber den Erträgen der Parzelle 4 (Mineralsalze ohne Stickstoff) betragen in Kilogramm¹⁾:

	Der je Hektar zugeführte Stickstoff in Kilogramm		Gesamtertrag				Kornenertrag			
	Bis 1906 ²⁾	Nach 1906	Erster Zeitraum 8 Jahre 1889 bis 1896	Zweiter Zeitraum 10 Jahre 1897 bis 1906	Letzter Zeitraum 20 Jahre 1907 bis 1926	Erster Zeitraum 8 Jahre 1889 bis 1896	Zweiter Zeitraum 10 Jahre 1897 bis 1906	Letzter Zeitraum 20 Jahre 1907 bis 1926		
Rapskuchen, Parzelle 10 b . .	91,8	23,0	27	37	52	12	13	22		
Stallmistdüngung, Parzelle 11 b	123,2	91,8	21	25	35	8	9	11		
Natronsalpeter + Mineralsalze, Parzelle 6	45,9	23,0	62	61	65	24	24	25		
Die Wirkung des im Raps- kuchen enthaltenen Stick- stoffs (der in Natronsalpeter enthaltene Stickstoff = 100)	—	—	14	61	80	50	54	88		
Entsprechende Wirkung bei Gerste (hier findet die Raps- kuchendüngung später statt)	—	—	38	38	—	50	60	—		

Nach 1906 wurde die Rapskuchendüngung auf $\frac{1}{4}$ ihrer früheren Menge reduziert: Je Hektar 25,8 kg Stickstoff anstatt 91,8 kg. Darauf erfolgte sofort ein Absinken der Erträge, das in keiner Weise der Verminderung der Düngung entsprach. Von da an erzielte man mit den abgeschwächten Düngungen zwar je Kilogramm zugeführten Stickstoffs eine Ertragssteigerung; aber aus dieser Tatsache lassen sich keine weiteren Schlüsse ziehen, weil ein unbekannter Teil der Wirkung den sich aus der Düngung ergebenden Restbeständen möglicherweise zugeschrieben werden muß. In jedem folgenden, fünf Jahre umfassenden Zeitraum ließ die Wirksamkeit des Stickstoffs nach und erreichte schließlich die Wirkung

¹⁾ Angaben über die Düngung vor und nach der Reduzierung der Gaben:

Ertragssteigerungen je Kilogramm zugeführten Stickstoffs.

	10 Jahre 1890 bis 1899	7 Jahre 1900 bis 1906	5 Jahre 1907 bis 1911	5 Jahre 1912 bis 1916	5 Jahre 1917 bis 1921	5 Jahre 1922 bis 1926
Gesamtertrag.						
Rapskuchen						
92 kg Stickstoff . .	32	35	—	—	—	—
23 kg Stickstoff . .	—	—	85	66	37	21
Stallmistdüngung						
124 kg Stickstoff . .	23	24	—	—	—	—
62 kg Stickstoff . .	—	—	44	33	40	22
Kornenertrag.						
Rapskuchen						
92 kg Stickstoff . .	12	13	—	—	—	—
23 kg Stickstoff . .	—	—	35	28	16	9
Stallmistdüngung						
124 kg Stickstoff . .	8	9	—	—	—	—
62 kg Stickstoff . .	—	—	14	11	15	5

²⁾ Ausgenommen 1889, in dem Jahre wurde nur 46 kg gegeben.

des in Stallmistdüngung vorhandenen Stickstoffs; die Wirkung blieb bei Weizen immer noch weit höher als bei Gerste. Aus irgend einem bis jetzt noch unerklärten Grunde wurde der Boden der mit Rapskuchen gedüngten Parzellen sehr sauer, eine Tatsache, die sich naturgemäß bei Gerste mehr bemerkbar machte als bei Weizen.

7. Das Verhältnis von Kornertrag zu Gesamtertrag.

Beim Weizen ist die Trennung des Pflanzenmaterials in Korn und Stroh nicht so genau durchgeführt worden wie bei Gerste: bei Weizen gehören zwischen 35 und 40 % der gesamten Pflanzensubstanz zum Korn, bei Gerste dagegen ungefähr 45 % (Tabelle 31). Folglich liegen bei den Versuchen in Woburn die Kornerträge bei Weizen niedriger als bei Gerste.

Wie bei Gerste wird auch hier das Verhältnis von Kornertrag zu Gesamtertrag durch die Düngung nicht wesentlich beeinflusst. Es ist am höchsten bei den ungedüngten Parzellen, bei denjenigen, die mit schwefelsaurem Ammoniak (ausgenommen in stärksten Dosen) und Rapskuchen gedüngt worden sind, sowie bei der Parzelle ohne Kaligaben (10 b). Die Versäuerung der mit schwefelsaurem Ammoniak und Rapskuchen gedüngten Parzellen sowie der Kalimangel der Parzelle 10 a scheint sich auf den Strohertrag nachteiliger auszuwirken als auf den Kornertrag. Das Verhältnis nimmt ab, wenn die Parzellen mit Stallmist, nur mit Mineralsalzen, mit Natronsalpeter und mit sehr hohen Gaben von schwefelsaurem Ammoniak gedüngt werden. Bis auf den letzten, fünf Jahre umfassenden Zeitraum, in dem bei allen Parzellen — mit Ausnahme einer einzigen — eine beträchtliche Ertragsdrückung einsetzte, konnte man in dem Verhältnis Kornertrag zu Gesamtertrag keine Neigung zum gleichmäßigen Abfallen beobachten. Auf Parzelle 11 a (Stickstoff und Kali, aber keine Phosphorsäure) machte sich das Absinken am deutlichsten bemerkbar; diese Kombination hielt den Strohertrag mehr auf derselben Höhe als den Kornertrag.

Tabelle 43.

Das Verhältnis Kornertrag/Strohertrag und das Verhältnis Kornertrag/100 Teile Gesamtertrag auf Weizenparzellen (Broadbalk).

	Kornertrag zu 100 Teilen Gesamtertrag		
	Parzelle 3 Ungedüngt	Parzelle 2 B Stallmist- düngung	Parzelle 7 Volldüngung
8 Jahre (1844 bis 1851)	38,8	38,2	37,7
10 Jahre (1852 bis 1861)	36,9	36,1	34,6
10 Jahre (1862 bis 1871)	41,6	38,5	37,4
10 Jahre (1872 bis 1881)	40,2	36,8	34,8
10 Jahre (1882 bis 1891)	44,8	37,9	36,5
10 Jahre (1892 bis 1901)	43,8	37,0	37,1
10 Jahre (1902 bis 1911)	40,1	33,8	34,0
10 Jahre (1912 bis 1921)	40,6	33,9	35,2
10 Jahre (1922 bis 1931)	36,1	28,6	29,4
1932	31,8	15,2	20,9

In Woburn ist das Verhältnis von Kornertrag zu Gesamtertrag demjenigen ähnlich, das man auf dem Broadbalk-Acker in Rothamsted bei Weizen feststellen konnte (Tabelle 43). Bei den ungedüngten Pflanzen in Rothamsted ist das Verhältnis höher als in Woburn; aber der Unterschied machte sich hauptsächlich in den Jahren 1882 bis 1901 bemerkbar, in welchen das Verhältnis in Rothamsted auch besonders hoch war. Bei den Pflanzen, die Stallmistdüngung und künstliche Volldüngung erhielten, sind die Werte auf beiden Stationen annähernd gleich, aber sie liegen an beiden Orten niedriger als bei ungedüngten Parzellen.

Sowohl in Rothamsted als auch in Woburn hielt sich das Verhältnis während einer ganzen Reihe von Jahren auf derselben Höhe; nach einem bestimmten Zeitpunkt aber setzte das Abfallen ein. Bei Gerste begann das Absinken früher als bei Weizen.

8. Jährliche Ertragsschwankungen bei Weizen.

Die jährlichen Ertragsschwankungen sind durch die Methoden von R. A. Fisher analysiert und die einzelnen Ergebnisse sind von W. G. Cochran besprochen worden.¹⁾

Die geringste Schwankung („variance“) liegt ungefähr bei 9% und wurde auf Parzelle 9 (Künstliche Volldüngungen mit 92 kg je Hektar Stickstoff als Natronsalpeter) festgestellt; hiernach kommt Parzelle 11 b (Stallmistdüngung); dann folgt Parzelle 6 (künstliche Volldüngung mit 46 kg je Hektar Stickstoff als Natronsalpeter), bei welcher die Schwankung auf annähernd 13% stieg. Auf allen diesen Parzellen ist die Schwankung hauptsächlich auf Jahresursachen, wie auf das Wetter zurückzuführen, weniger auf langsame Veränderungen und am wenigsten auf Bodenverschlechterung, die bei Parzelle 11 b (Stallmist) kaum in Frage kommt.

Eine größere Schwankung, die ungefähr 20% beträgt (18 bis 23%) kann man bei den ungedüngten Parzellen und denjenigen ohne Volldüngung feststellen, z. B. bei der nur mit Stickstoff (Parzelle 3) oder nur mit Mineralsalzen (Parzelle 4) gedüngten Parzelle oder bei den Parzellen, die künstliche Volldüngung, aber den Stickstoff als schwefelsaures Ammoniak erhalten haben (Parzelle 5). Verschiedene Ursachen rufen eine Zunahme der Schwankungen hervor: Bei den Parzellen ohne Stickstoff und denjenigen mit stärkeren Gaben von schwefelsaurem Ammoniak ist zu einem hohen Maße — bis zu 50% — die Bodenverschlechterung verantwortlich zu machen; dagegen ist kein Anzeichen für langsame Änderungen vorhanden.

Die größte Schwankung, 50%, zeigt sich auf der nur mit schwefelsaurem Ammoniak gedüngten Parzelle; Jahresursachen bilden die Hälfte der hierfür verantwortlichen Faktoren, während die Bodenverschlechterung den Hauptanteil der zweiten Hälfte darstellt.

In allen Fällen ist in Woburn die jährliche Schwankung größer als auf dem Broadbalk-Feld in Rothamsted. Der Hauptunterschied liegt in dem Einfluß der Jahresursachen, der in Woburn eine weit wichtigere Rolle

¹⁾ 50 Jahre Feldversuche der Versuchsstation Woburn. Von E. John Russell und J. A. Voelcker. Mit einem statistischen Bericht von W. G. Cochran.

spielt als in Rothamsted. In Woburn macht sich auch bei einzelnen Parzellen, wie bei denen ohne Stickstoffdüngung (ungedüngt oder nur Mineralsalzdüngung) und teilweise auch bei der nur mit schwefelsaurem Ammoniak gedüngten Parzelle eine Bodenverschlechterung deutlicher bemerkbar als in Rothamsted, eine Tatsache, die sowohl durch direkte Feststellung der Erträge in Doppelzentnern je Hektar als auch durch das Berechnen der prozentischen Werte bewiesen ist.

Die Werte betragen:

	Rothamsted			Woburn 1877 bis 1906		
	Durchschnittlicher Ertrag in Doppelzentnern je Hektar	Durchschnittliche jährliche Abnahme		Durchschnittlicher Ertrag in Doppelzentnern je Hektar	Durchschnittliche jährliche Abnahme	
		in Doppelzentnern je Hektar	in Prozenten		in Doppelzentnern je Hektar	in Prozenten
Ungedüngt.	8,25	0,065	0,79	9,06	0,234	2,58
Nur Mineralsalze	9,54	0,060	0,63	8,66	0,293	3,39
Mineralsalze + 1 Teil Stickstoff . . .	15,19	0,095	0,62	19,32	0,278	1,44
Mineralsalze + 2 Teile Stickstoff . .	21,10	0,097	0,46	21,77	0,253	1,16
Mineralsalze + 3 Teile Stickstoff . .	24,00	0,062	0,26	—	—	—
Stallmistdüngung	23,23	0,021	0,09	17,58	0,000	0,00

Weizen zeigt in Woburn eine größere Gesamtschwankung als Gerste; eine Ausnahme bilden die Parzellen ohne Stickstoff (ungedüngt und nur Mineralsalzdüngung), bei welchen kein Unterschied ist, und die mit schwefelsaurem Ammoniak gedüngten Parzellen, auf denen Gerste eine erheblich größere Schwankung zeigt. Im Gegensatz zu dem, was man hätte erwarten sollen, sind die Jahreseinflüsse bei Weizen ausgeprägter als bei Gerste.

9. Einfluss der Niederschläge auf Weizenernten.

Für Weizen wurde diese Untersuchung nach derselben Methode durchgeführt wie für Gerste. Die Kurven, die die geradlinigen Wirkungen der Niederschläge auf den verschiedenen Parzellen angeben, verlaufen im ganzen ziemlich ähnlich. Nur die Stallmistparzelle und diejenige, die künstliche Volldüngung mit doppelter Menge schwefelsaurem Ammoniak erhalten haben (Parzelle 8), weichen von den übrigen ab.

Bei allen anderen Parzellen zeigen sich September- und Oktober-Niederschläge schädlich, Januar-Regen begünstigt die Erträge, Niederschläge im Mai und Juni haben schädigenden Einfluß. Düngungen mit Mineralsalzen scheinen eine größere Stetigkeit zu bewirken, denn die Schwankungen sind bei den Parzellen 6 und 9 geringer als bei Parzelle 3. Wie bei Gerste, so wirkt sich auch bei Weizen ein Überschuß an Niederschlägen auf Parzellen mit Stallmistdüngung immer sehr schädlich aus; die wasserhaltende Kraft des Stallmistes scheint in diesem Fall ein Nachteil zu sein. Parzelle 8 dagegen (doppelte Gaben von schwefelsaurem Ammoniak und

Mineralsalzen) wird als einzige von allen Versuchspartzen durch einen Überschuß an Regen günstig beeinflusst, zweifellos deshalb, weil der durch die Versäuerung hervorgerufene Schaden unter feuchten Bedingungen geringer ist als bei Trockenheit. Die Unterschiede sind nicht groß genug, um nach den üblichen statistischen Methoden als gesichert zu gelten, aber sie stimmen mit den bekannten Eigenschaften der Düngungen überein. Die geradlinigen Wirkungen der Niederschläge sind für Weizen geringer als für Gerste, gleichgültig, ob dem Vergleich die absoluten Zahlen in Doppelzentnern je Hektar oder die prozentischen Erträge zugrunde gelegt werden, und die Maxima und Minima der Kurven liegen für die beiden Getreidearten in verschiedenen Jahreszeiten (Seite 39).

In Rothamsted sind die geradlinigen Wirkungen nicht nur statistisch gesichert und ausgeprägter als in Woburn, sondern sie zeigen sich auch zu verschiedenen Jahreszeiten. In Rothamsted wirken sich weitere Niederschläge im Oktober günstig, im Januar schädlich, im April und Mai günstig, im Juli schädlich aus. Ähnlich wie in Woburn unterscheidet sich auch die Stallmist-Parzelle von den übrigen.

III. Die Möglichkeit, den Ausfall der Getreideernten vorauszusagen.

Die Entwicklung der für die Ergebnisse der Feldversuche benutzten statistischen Methoden erschließt die Möglichkeit, die Getreideernten bis zu einem bestimmten Grad der Genauigkeit vorauszusagen. In demselben Grade, wie die Entwicklungsgeschichte der Pflanze von einem gewissen Faktor abhängig ist, der früher in ihrem Leben eine Rolle gespielt hat, so besteht hier immer die Möglichkeit, aus einem bekannten Vorgang das Ergebnis vorauszusagen.

Wenn die Wirkungen der Niederschläge auf die Weizenерträge in Rothamsted, wie R. A. Fisher gezeigt hat, in beträchtlichem Grade durch polynomische Methoden ausgedrückt werden können, läßt sich naturgemäß annehmen, daß andere Pflanzen ähnlich darstellbare Beziehungen zeigen können. Dieses war nicht der Fall. Bei der Anwendung dieser Methode ließen sich in Rothamsted weder bei Gerste noch Mangold, in Woburn weder bei Weizen noch Gerste deutliche Einflüsse von Niederschlägen oder anderen meteorologischen Vorgängen feststellen. In allen diesen Fällen ist die Wirkung der Niederschläge komplizierter als bei Weizen auf dem Broadbalk-Acker und kann nur durch Benutzung kubischer und quadratischer Zeichen ausgedrückt werden. Dieses Problem muß ständig weiter erforscht werden, da bei der Bestimmung der Ernten die Witterungseinflüsse eine große Rolle spielen. Es kann aber gut möglich sein, daß die von uns angewandte Methode nicht den geeigneten Maßstab darstellt zur Erfassung der klimatischen Faktoren, die für den Pflanzenbau von besonderer Bedeutung sind.

Eine weitere Möglichkeit der Voraussage bildet die auf Seite 67 besprochene enge Beziehung zwischen Kornertrag und Gesamtertrag. Die Entwicklungsgeschichte der Pflanze zerfällt in zwei Abschnitte: Zuerst die Bildung des Pflanzenmaterials, der Kohlehydrate, der Proteine usw.,

dann die Einlagerung eines Teiles dieser Substanz in das Korn. Diese beiden Stadien greifen ineinander über; kurz vor der Schnittreife des Kornes ist die Bildung des Pflanzenmaterials praktisch beendet. Es sollte möglich sein, aus einer richtigen Schätzung der Gesamtproduktion eine richtige Schätzung des Kornertrages abzuleiten. Dieses Problem wird augenblicklich im einzelnen in Rothamsted behandelt. Fräulein Mildred Barnard hat bereits gezeigt, daß die Pflanzenlänge ein gutes Kennzeichen für die Grundlage darstellt, auf die sich die Voraussage aufbaut.

Tabelle 44.

Durchschnittlicher, jährlicher für Korn und Stroh festgestellter Stickstoffgehalt in Kilogramm je Hektar.

Parzelle	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926	Ge- samt 1882 bis 1926
Gerste										
1 und 7	33	29	25	20	20	13	16	15	9	900
4 a	29	32	33	25	23	14	24	19	13	1060
2 a	58	65	53	14	2	—	—	—	—	—
3 a	63	56	58	47	40	30	24	22	20	1800
5 a	61	64	67	19	7	—	—	—	—	—
6	75	67	73	61	52	29	33	28	23	2205
9 mit Stickstoff	100	86	95	84	80	44	37	35	30	2955
9 ohne Stickstoff	54	45	52	37	34	24	25	22	18	1555
11 b	79	54	72	58	59	49	44	48	31	2470
Weizen										
1 und 7	37	30	22	21	20	21	19	19	8	990
4 a	39	30	20	19	18	19	16	21	10	964
3 a	60	52	40	45	36	37	27	31	18	1726
6	80	68	54	60	50	38	31	40	20	2220
11 b	70	66	49	54	54	46	37	49	20	2224

1. Die Zusammensetzung des Getreidekornes.

Gerste.

Chemische Kornuntersuchungen sind im Laufe der Versuchszeit nicht durchgeführt worden. Aber es war günstig, daß von den meisten der Parzellen Sortimente noch vorhanden waren: ihr Stickstoffgehalt und ihr 1000-Korngewicht sind bestimmt worden. Gelegentlich seiner in Rothamsted nach dem Versuchsplan des Instituts für Brauereiwesen durchgeführten umfangreichen Studien über Gerste prüfte L. R. Bishop zahlreiche Gerstenproben aus Woburn: abgesehen von einem ziemlich hohen Stickstoffgehalt fand er nichts Außergewöhnliches an ihnen. Die mutmaßliche durchschnittliche Zusammensetzung des Kornes kann man aus diesen Angaben ungefähr schätzen:

Ungefährer Prozentsatz im trockenen Korn.

Festgestellter Stickstoff in Prozenten	Gesamte Stickstoff- verbindungen	Hordein	Glutelin	Salzlosliche Bestandteile	Hydrolysierbare oder Extrakt liefernde Kohlehydrate
1,9	11,4	4,3	4,1	3,0	73

Tabelle 45.
Stickstoffgehalt in Prozenten, berechnet aus retrokometrierter bei 1000 °C.

Parzelle	1877	1878	Erster Zeitraum 1882 bis 1891	Zweiter Zeitraum 1892 bis 1906	Dritter Zeitraum 1907 bis 1926	Mittel 1882 bis 1926						
1 und 7	1,68	1,67	1,98 ²⁾	1,82	1,87 ²⁾	1,91	1,76	1,87	1,80	1,84	1,76	1,82
4 a	1,38	1,54	1,74	1,69	1,70 ¹⁾	1,79 ²⁾	1,63	1,75	1,74	1,82	1,80	1,74
2 a	1,60	1,78	1,82 ¹⁾	2,16 ²⁾	2,00 ¹⁾	2,25	2,06	1,69 ¹⁾	1,93	1,97	1,72 ²⁾	2,00
3 a ¹⁾	1,72	1,85	2,02 ²⁾	2,00 ¹⁾	2,05 ³⁾	2,20	2,00	1,98	2,06	2,06	2,06	2,05
5 a	1,65	1,60	1,76 ³⁾	1,85	2,06 ³⁾	2,29 ¹⁾	1,86	1,76 ²⁾	2,00 ¹⁾	1,90 ³⁾	1,80	1,92
6	1,64	1,78	1,64 ¹⁾	1,81	1,92 ²⁾	1,94	1,78	1,77	1,87	1,83	1,88	1,84
8 a Mit Stickstoff . .	1,74	1,96	—	—	—	—	—	—	—	—	—	—
Ohne Stickstoff . .	—	—	—	—	—	—	—	—	—	—	—	—
9 a, b Mit Stickstoff . .	1,79	2,08	1,98 ²⁾	2,15	2,07 ²⁾	2,34	2,05	1,82	1,89	1,94	2,03	2,03
Ohne Stickstoff . .	—	—	2,06 ²⁾	1,76	1,64 ¹⁾	1,84 ³⁾	1,71	1,74	1,75	1,82	1,64 ³⁾	1,81
10 a	—	—	—	—	—	—	—	1,78	1,88	1,90	1,86	—
10 b	1,59	1,69	—	—	—	—	—	—	—	—	—	—
11 a	—	—	—	—	—	—	—	1,82	1,87	1,91	1,98 ³⁾	—
11 b	1,66	1,64	2,09 ²⁾	1,77	1,81 ¹⁾	1,84	1,74	1,77	1,73	1,96	1,90 ³⁾	1,83

¹⁾ 3b von 1902 bis 1926.

²⁾ Diese Zahl stellt das Mittel von weniger als fünf Jahren dar.

Stickstoffgehalt des Korns.

Für die wichtigeren Parzellen ist in Tabelle 45 der prozentische Stickstoffgehalt des Korns angegeben. Der Mittelwert 1,88 liegt deutlich höher als die Werte, die man auf guten Gerstenböden erhält (sie liegen hier gewöhnlich zwischen 1,4 und 1,55), aber er ist für sandige Böden nichts Außergewöhnliches. Der Mittelwert ist auch etwas höher als der aus den Fruchtfolgeversuchen in Woburn, aber die Angaben sind zu lückenhaft, so daß in dieser Richtung noch keine allgemein gültige Schlußfolgerung gezogen werden darf.

	1922	1923	1924	1925	1926	Mittel
Dauer-Gerstenbau (Parzelle 6)	1,65	2,07	1,44	2,29	1,96	1,88
Gerste im Fruchtwechsel	1,95	1,71	1,23	2,01	1,57	1,69

(Versuchsreihen des Instituts für Brauereiwesen.)

Durch diesen hohen Prozentsatz an Stickstoff waren die Gersten von diesen Parzellen in Woburn niemals für Mälzereizwecke geeignet, nicht einmal in den achtziger Jahren, als die Parzellen mit Dauergerstenbau in Rothamsted Gersten von guter Brauqualität lieferten.

Der Stickstoffgehalt des Korns hat im Gegensatz zum Gesamtertrag im Laufe der Jahre keine Tendenz zu Veränderungen gezeigt. Diese Beobachtung weicht sehr von der auf dem Hoos-Feld in Rothamsted festgestellten ab, wo mit fortschreitender Ertragsminderung der Stickstoffgehalt des Korns zu steigen pflegte. Wenn diese Tendenz auch in Woburn vorhanden wäre, würde sie auf jeden Fall durch die Tatsache verdeckt werden, daß die Gerstensorte „Plumage Archer“, die während der letzten fünf Versuchsjahre angebaut worden war, im Vergleich zu den älteren Sorten gewöhnlich Korn mit niedrigerem Stickstoffgehalt liefert. Die jährlich festgestellten, prozentischen Schwankungen sind für den Stickstoffgehalt weit geringer als für den Ertrag, sie betrugen nur 10 bis 15 %, während sie bei den Erträgen 40 bis 50 % ausmachen (Tabelle 46).

Einfluß der Düngung auf den Stickstoffgehalt des Korns.

Der durchschnittliche Stickstoffgehalt ist für ungedüngte Gerste 1,82 %; durch Mineralsalzdüngungen sinkt er, durch stickstoffhaltige Düngemittel steigt er. Bei kombinierten Düngungen mit 46 kg Stickstoff je Hektar (Parzelle 6) sind diese Einflüsse fast genau ausgeglichen, so wie es bei der Stallmistparzelle der Fall ist. Die stärkeren Stickstoffgaben (92 kg je Hektar) auf den Parzellen 9 steigerten den Stickstoffgehalt des Korns bis zur Höhe der Parzelle, die 46 kg Stickstoff je Hektar, aber keine Mineralsalze erhalten hatte, so daß man bei den beiden Parzellen 6 und 9 den Eindruck hat, als ob die Gaben von 4,4 dz je Hektar Superphosphat und 2,2 dz je Hektar Kalisulfat¹⁾ die Wirkung von 46 kg je Hektar

¹⁾ Je Hektar waren 1,1 dz einer Mischung von Natronsulfat und Magnesiumsulfat zur Anwendung gekommen, aber es ist nicht bewiesen, daß diese Zusammensetzung das Ergebnis beeinflusste.

als Natronsalpeter oder schwefelsaures Ammoniak zugeführten Stickstoffs aufheben würden. Für den Zeitraum von 1882 bis 1906 sind die Zahlen fast die gleichen wie für die Zeit von 1907 bis 1926. Das Ergebnis von Rothamsted ist in der Hauptsache das gleiche, abgesehen davon, daß die Stallmist-Parzellen eine besondere Stellung einnehmen. Stickstoffhaltige Düngemittel allein steigern den Stickstoffgehalt des Korns; werden sie aber mit Mineralsalzen zusammen angewandt, so wird der Stickstoffgehalt fast bis auf den Stand der ohne Stickstoff behandelten Parzelle gesenkt, und wiederum haben 4,4 dz Superphosphat und 2,2 dz Kalisulfat¹⁾ die Wirkung von 46 kg mit der Düngung zugeführten Stickstoffs beinahe ausgeglichen.

Von Angehörigen der Versuchsstation in Rothamsted nach den Richtlinien des Instituts für Brauereiwesen exakt durchgeführte Versuchsserien mit Gersten aus verschiedenen Teilen des Landes ergaben ähnliche Ergebnisse, abgesehen davon, daß die zahlenmäßigen Werte niedriger lagen.

Tabelle 46.
Durchschnittlicher prozentischer Stickstoffgehalt
bei Gerste, die unter verschiedenen Verhältnissen gewachsen war.

Düngung	Parzelle	Woburn		Rothamsted Hoos-Field	Dem Institut für Brauerei- wesen aus verschiedenen Gegenden zugegangene Braugersten
		1885 bis 1906	1906 bis 1926		
Ungedüngt	1 und 7	1,83	1,82	1,47	1,47
Nur Mineralsalze	4	1,70	1,78	1,51	1,46
Nur Stickstoff	2, 3b, 3a	2,06	2,00	1,67	1,50
Mineralsalze + 46 kg Stickstoff	6	1,83	1,81 ²⁾	1,51	1,47
Stallmistdüngung	11b	1,83	1,81 ²⁾	1,81	-
Mineralsalze + 92 kg Stickstoff	9	2,15	1,92 ²⁾	-	-

Es ist kein Anzeichen für irgendwelche, durch stickstoffhaltige Düngemittel verursachte Abnahme im Stickstoffgehalt des Korns vorhanden, wie man sie häufig beobachtet, wenn die Düngemittel reduziert werden und die gewöhnliche Höhe des Stickstoffgehaltes niedriger wird. Sogar die Nachwirkungen von Natronsalpeter steigerten den prozentischen Stickstoffgehalt des Korns, wie aus nachstehenden Mittelwerten ersichtlich ist:

Ständig ohne Stickstoff, nur Mineralsalze Parzelle 4	Nur im vorhergehenden Jahr Stickstoff, im laufenden nur Mineral- salze Parzelle 9b	Im laufenden Jahr sowohl Stickstoff als auch Mineralsalze Parzelle 9a
1,74	1,81	2,03

¹⁾ Siehe ¹⁾ auf S. 73.

²⁾ Verminderte Düngung.

Die allgemeine Ansicht, daß der Stickstoffgehalt gesenkt wird, wenn man statt mit Natronsalpeter mit schwefelsaurem Ammoniak düngt, ist hierdurch nicht bewiesen. Bei zunehmender Versäuerung des Bodens tritt keine Erhöhung des Stickstoffgehaltes ein; bei Kalkdüngung wird der Stickstoffgehalt niedriger, aber nur auf zwei Parzellen, nicht auf der dritten.

Die Wirkungen sind alle gering; die bis jetzt durchgeführten Düngungspläne haben nicht viel mehr erreicht, als die Einwirkung der stickstoffhaltigen Düngung auszugleichen. Wie für alle anderen Früchte, soweit sie untersucht worden sind, steigert die Düngung den Ertrag, aber beeinflußt kaum die Qualität. Solange Düngungen die Erträge steigern und kein Lager verursachen, besteht auch hier kein Grund, ein Nachlassen in der Qualität zu befürchten, man könnte sogar eine, wenn auch geringe Verbesserung erwarten. Aber eine besondere Veränderung findet nicht statt. Durch die Düngung kann der Stickstoffgehalt nicht auf den Stand der Braugerstenreihen gesenkt werden. Der Stickstoffgehalt ist in hohem Maße abhängig von der allgemeinen Bodenbeschaffenheit und den klimatischen Bedingungen; er kann zwar durch Düngungen gesteigert, aber nicht leicht wieder gesenkt werden. Es besteht tatsächlich keine Möglichkeit, eine Stickstoffsenkung mit Sicherheit zu erreichen.

Ursachen der Jahresschwankung im Stickstoffgehalt des Korns

Beziehungen zwischen Stickstoffgehalt und Ertrag.

Auf allen Parzellen hatten hohe Erträge niedrigeren Stickstoffgehalt zur Folge; jedes weitere Hektoliter Korn je Hektar verminderte den Stickstoffgehalt um ungefähr 0,01 %.

Tabelle 47
Ein Vergleich der Schwankungen von Stickstoffgehalt
des Gerstenkorns und Kornertrag.

Düngung	Parzelle	Zahl der Jahre ¹⁾	Stickstoffgehalt des trockenen Korns in Prozenten ²⁾		Schwankung in Prozenten	
			Durchschnitt	Jährliche Schwankung	Beim Stick- stoffgehalt	Beim Ertrag
Ungedüngt	1	40	1,82	+ 0,18	10	49
Nur Mineralsalze ohne Stickstoff	1	37	1,74	+ 0,21	12	39
Nur Natronsalpeter	3 a	37	2,05	+ 0,25	12	38
Nur schwefelsaures Ammoniak	2 a	30	2,00	+ 0,24	12	—
Mineralsalze + Natronsalpeter	6	39	1,84	+ 0,27	15	26
Mineralsalze + schwefels. Ammoniak	5 a	35	1,92	+ 0,25	13	—
Stallmistdüngung	11 b	40	1,83	+ 0,22	12	26

¹⁾ Die Zahlen für verschiedene Jahre fehlen bei verschiedenen Parzellen, so daß während der 44 Jahre, von 1883 bis 1926, für keine Parzelle vollständige Zahlenreihen zur Verfügung stehen. Die erwähnten Zahlen sind aus allen zugänglichen Jahren übernommen worden.

²⁾ Standardfehler der Jahreszahlen.

Wirkung der Niederschläge.

Auf den Stickstoffgehalt des Korns haben Niederschläge einen viel größeren Einfluß als auf den Ertrag. Die Kurve der Abb. 9, die diese Wirkung zeigt, genügt den statistischen Ansprüchen auf Sicherheit, während das bei Abb. 5, die den Ertrag darstellt, kaum der Fall ist. Die für den Stickstoffgehalt kritischste Niederschlagsperiode ist Mai, Juni, Juli. Anfang Juni 25 mm Niederschläge über dem Durchschnitt senken den Stickstoffgehalt um ungefähr 0,10 %. Es ist sehr auffallend, daß diese für den Stickstoffgehalt so wichtigen Juni-Niederschläge auf den Ertrag eine geringe Wirkung haben, während die Niederschläge im März und April die Erträge vermindern, den Stickstoffgehalt aber nur wenig beeinflussen und ihn dann sogar zu steigern pflegen.

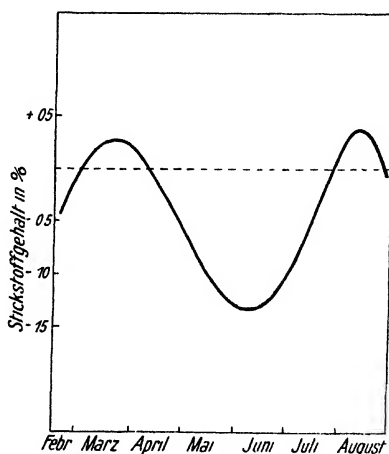


Abb. 9

Kurve für mittleren Gerstenertrag auf sieben Parzellen.

Wirkung von 25 mm Niederschlägen über dem Durchschnitt auf den Stickstoffgehalt.

Eine Einwirkung der Düngung läßt sich nicht feststellen, alle Parzellen verhalten sich gleich. Der enge Zusammenhang zwischen Frühsommerniederschlägen und Stickstoffgehalt des Korns ist nicht auf Woburn beschränkt, er wird auch von den anderen Stellen beobachtet, die die Gersten-Versuche des Instituts für Brauereiwesen durchführten. Diese Feststellung läßt sich wahrscheinlich damit erklären, daß das in den ersten Wachstumswochen von den Pflanzen aufgenommene Nitrat eher dazu neigt, eine Steigerung des Stickstoffgehalts im Korn als eine erhöhte Kornbildung herbeizuführen. Frühsommer-Niederschläge, die aus dem Boden das spät gebildete oder überschüssige Nitrat auswaschen, verhindern diese Vermehrung des Stickstoffgehalts. Es ist oft festgestellt worden, daß Niederschläge im Juli oder im frühen August für die Senkung des Stickstoffgehalts eine wichtige Rolle spielen, ein Beweis dafür ist aber nicht vorhanden. Die Reife und damit die Qualität des Korns wird durch Juli-Regen günstig beeinflusst.

Einfluß der Temperatur.

Bis Mitte Mai (d. h. während der ersten acht oder zehn Wochen nach der Aussaat) haben Temperatur-Schwankungen nur wenig Einfluß auf den Stickstoff-Gehalt des Korns, abgesehen davon, daß bei Temperatur-Steigerungen auf den Parzellen mit künstlicher Volldüngung ein leichtes Absinken eintritt.

Von der zweiten Mai-Hälfte an steigern überdurchschnittliche Temperaturen den Stickstoffgehalt. Die Wirkung hängt in gewisser Beziehung von der Düngung ab, dadurch unterscheidet sie sich von dem Einfluß der Niederschläge. Bei den Stallmist-Parzellen zeigt sich diese Wirkung am wenigsten ausgeprägt, bei den Natronsalpeter-Parzellen am deutlichsten. Die empfindlichste Periode ist Mitte Juli; eine um 5° C über dem Durchschnitt liegende Temperatur kann den Stickstoffgehalt wie folgt steigern:

Stickstoffsteigerung in Prozenten	Parzelle	Düngung
0,0076	1, 7	Ungedüngt
0,0128	3, 6	Natronsalpeter
0,0083	5	Schwefelsaures Ammoniak
0,0059	11 b	Stallmistdüngung

Der Einfluß der Temperatur kann nicht genau von dem der Niederschläge getrennt werden, weil feuchte Tage im Sommer oft kühler sind als trockene.

Zusammenwirken von Niederschlägen und Temperatur.

Tabelle 48 zeigt, daß die Niederschläge im Mai und Juni und die Temperatur im Juli zu etwa 33 bis 60 % für die Jahresschwankungen verantwortlich zu machen sind.

Tabelle 48.

Anteil des Witterungseinflusses
an der Jahresschwankung im Stickstoffgehalt in Prozenten.

Regression des prozentischen Stickstoffgehaltes aus den Mai- und Juni-Niederschlägen und dem Maximum der Juli-Temperatur.

Kein Stickstoff			Natronsalpeter				Stallmist- düngung	
	Par- zelle	%		Par- zelle	%		Par- zelle	%
Ungedüngt . . .	1	33	Allein	3a	57		11b	42
Ungedüngt . . .	7	43	+ Mineralsalze	6	60			
Mineralsalze allein	4a	33	Schwefelsaures Ammoniak + Mineralsalze	5a	39			

Die Voraussage des Stickstoffgehaltes.

Der Stickstoffgehalt steht so eng mit Frühsommerregen und Juli-Temperatur in Zusammenhang, daß eine sichere „Voraussage“ des Stickstoffgehaltes des Korns für die ganze Zeit aus der Kenntnis dieser beiden Größen konstruiert werden kann. Tabelle 49 zeigt die „Voraussage“ für Parzelle 6: Nicht weniger als 75 % der Ergebnisse wurden gefunden, bei

welchen sich nur eine Abweichung um 0,15 % ihrer tatsächlichen Werte feststellen ließ¹⁾.

Wie Bishop gezeigt hatte, ergaben sich hieraus interessante Möglichkeiten insofern, als die Voraussage des Stickstoffgehaltes auch zu einer Voraussage hinsichtlich der allgemeinen chemischen Zusammensetzung des Korns und der Menge des sich nach dem Vermälzen ergebenden Malzextraktes führen kann²⁾.

Einfluß der Anbau-Maßnahmen.

Aussaatzeiten. Februar ist im allgemeinen der beste Monat zur Aussaat der Gerste. Obgleich spätere Aussaattermine beim Auswählen eines günstigen Zeitpunktes den Ertrag nicht wesentlich beeinflussen, haben sie doch die Neigung, einen höheren Stickstoffgehalt zu bewirken. Jede Verzögerung der Aussaatzeit um eine Woche nach Ende Februar bedeutet eine Vermehrung des Stickstoffgehaltes im Korn um annähernd 0,05 %.

Zeitpunkte

für die Durchführung der stickstoffhaltigen Düngung.

Besondere Versuche wurden in Woburn in dieser Richtung nicht durchgeführt, aber das ständige Verschieben des Zeitpunktes, an dem die stickstoffhaltige Düngung zur Anwendung kam, steht im Zusammenhang mit einer nicht gleichmäßigen Veränderung im Stickstoffgehalt. Es ist möglich, daß hier, wie bei der Verschlechterung, eine Neigung zur Steigerung vorliegt, die aber erst beobachtet wurde, als die älteren Gerstensorten von 1922 an durch Plumage Archer ersetzt wurden.

Einfluß der Brache.

Eines der auffallendsten Versuchsergebnisse in Woburn war die außergewöhnliche Einwirkung der Brache auf Ertrag und Stickstoffgehalt

¹⁾ Die Berechnung wird für Parzelle 6 nach folgender Gleichung durchgeführt:

$$N = 0.396 \text{ TJY} - 0.11500 \text{ RM} - 0.0649 \text{ RJ}$$

wobei N die Abweichung des Stickstoffgehaltes im Korn vom Mittelwert bedeutet; TJY bedeutet die Abweichung der Maximaltemperatur im Juli vom Mittelwert in ° F; RM und RJ schließlich sind die Abweichungen der Niederschläge in Zoll im Mai bzw. im Juni von ihren Mittelwerten.

Die Werte der Koeffizienten für die einzelnen Parzellen sind die folgenden:

Düngung	Parzelle	TJY	RM	RJ
Ungedüngt	1	+ 0,026,310	-- 0,011,484	-- 0,020,829
Ungedüngt	7	+ 0,028,016	-- 0,056,394	-- 0,034,051
Nur Mineralsalze	4	+ 0,0229	-- 0,0525	-- 0,0628
Natronsalpeter				
Nur Stickstoff	3	+ 0,024,390	-- 0,168,458	-- 0,069,202
Natronsalpeter				
Künstliche Volldüngung	6	+ 0,0396	-- 0,1151	-- 0,0649
Schwefelsaures Ammoniak	5	+ 0,022,894	-- 0,097,068	-- 0,059,132
Stallmist	11 b	+ 0,0300	-- 0,0621	-- 0,0309

²⁾ Siehe E. J. Russell und L. R. Bishop. Investigations on barley J1. Inst. Brewing. 1933, Vol. 39. pp 287 et seq.

bei nachfolgendem Gerstenanbau. 1927 und 1928 wurden alle Parzellen brach gelassen, dann wurden sie mit Gerste bestellt, aber nicht gedüngt. Die Erträge stiegen in hohem Maße, aber der Stickstoffgehalt fiel. Nähere Angaben folgen: (Siehe auch Tabelle 55)

Ertrag und Stickstoffgehalt bei Gerste nach vorhergehender Brache.

	Erträge Doppelzentner je Hektar			Stickstoffgehalt		
	Vorhergehende Düngung			Vorhergehende Düngung		
	Nur Mineral- salze Parzelle 1	Stallmist- düngung Parzelle 11b	Un- düngt Parzelle 1 und 7	Un- gedüngt Parzelle 1 und 7	Stallmist- düngung Parzelle 11b	Nur Mineral- salze Parzelle 4
10-Jahresmittel vor der Brache (1917 bis 1926)	4,7	15,1	5,9	1,06	1,12	1,08
1. Jahr nach der Brache (1929)	11,8	20,2	12,3	0,78	0,77	0,75
2. Jahr nach der Brache (1930)	7,5	12,6	8,2	0,83	0,81	0,80

Tabelle 49.

Stickstoffgehalt des Gerstenkorns bei Parzelle 6, wie er durch Analyse festgestellt und durch Regression aus den Witterungsverhältnissen berechnet wurde.

Prozent des trockenen Korns

Jahr	Durch Analyse festgestellt	Ende Juli durch Regression von Mai- und Juni-Nieder- schlägen und Juli- Temperatur berechnet	Jahr	Durch Analyse festgestellt	Ende Juli durch Regression von Mai- und Juni-Nieder- schlägen und Juli- Temperatur berechnet
1885	1,73	1,77	1906	1,72	1,83
1886	—	1,72	1907	1,52	1,47
1887	1,81	1,70	1908	1,64	1,86 +
1888	1,81	1,78	1909	1,68	1,72
1889	1,67	1,53	1910	1,49	1,64
1890	1,85	1,83	1911	2,51	2,30 —
1891	1,93	1,60 —	1912	1,78	1,91
1892	1,66	1,93 +	1913	1,89	1,81
1893	—	2,01	1914	2,00	2,02
1894	1,73	1,78	1915	2,00	1,86
1895	2,38	2,27	1916	1,68	1,75
1896	—	2,16	1917	1,73	1,81
1897	1,90	2,00	1918	1,53	1,86 +
1898	1,56	1,66	1919	1,84	1,83
1899	1,87	2,09 +	1920	1,59	1,66
1900	1,99	2,10	1921	2,44	2,34
1901	2,36	2,12 —	1922	1,65	1,80
1902	1,63	1,68	1923	2,07	2,13
1903	1,60	1,39 —	1924	1,44	1,37
1904	1,83	2,06 +	1925	2,29	2,03 —
1905	2,10	1,99	1926	1,96	1,77 —

Anzahl der Fälle, in denen die berechneten Werte von den wirklichen Werten des Stickstoffgehalts um mehr als 0,15 abwichen = 11, = 25 %.

Diese Fälle sind bezeichnet mit +, wenn die Voraussage zu hoch war,

—, wenn die Voraussage zu niedrig war.

Seit 1926 wurde keine Düngung mehr gegeben, und aus dieser Tatsache allein hätte man schon eine Senkung des Stickstoffgehaltes im Korn erwarten können. Bei dem 10-Jahres-Durchschnitt hat das Fehlen der Düngung keinen solchen Unterschied hervorgerufen, wie dies bei der Brache der Fall war.

Die Weizen-Versuche.

Stickstoffgehalt des Kornes bei Weizen.

Wie in Rothamsted lag auch in Woburn der Stickstoffgehalt des Kornes bei Weizen ungefähr 0,2—0,3 % höher als bei Gerste. Die durchschnittlichen Werte bringt Tabelle 50.

Tabelle 50.

Durchschnittlicher prozentischer Stickstoffgehalt bei Weizen, der unter verschiedenen Bedingungen angebaut wurde.

	Parzellen	Woburn		Rothamsted Broadbalk	Gewöhnlicher englischer Weizen Durchschnitt der letzten Ergebnisse	Kanadischer ¹⁾	
		1885 bis 1905	1906 bis 1926			aus Prärie- provinzen	aus Küsten- provinzen
Ungedüngt . . .	1	2,02	1,87	1,85			
Nur Mineralsalze .	4	2,09	1,89	1,88			
Nur Stickstoff . .	3	2,20	1,95	2,07	2,0	2,0	2,1
Mineralsalze + Stickstoff, 46 kg	6	2,16	1,91	2,02			
Stallmistdüngung	11 b	2,11	1,97	2,21			

Der Stickstoffgehalt des in England angebauten Weizens zeigt starke Schwankungen (1,8—2,6 %): aber kürzlich durchgeführte Analysen ergaben einen Durchschnitt von 2,0 %²⁾, während Gerste im allgemeinen einen Stickstoffgehalt von ungefähr 1,4—1,8 % aufzuweisen hat. Während des ersten Zeitraumes hatte der Weizen in Woburn einen höheren Stickstoffgehalt als derjenige auf dem Broadbalk-Feld in Rothamsted, eine Beobachtung, die auch bei Gerste gemacht werden konnte und die übereinstimmte mit dem häufig festgestellten Ergebnis, daß das Korn auf sandigen Böden durchschnittlich reicher an Stickstoff ist als auf den schwereren Lehm Böden. In der zweiten Periode ist der Stickstoffgehalt beim Weizen auf beiden Stationen fast gleich, nur die Stallmistparzellen weichen ab. Der Weizen von Woburn steht dem gewöhnlichen englischen Weizen im Stickstoffgehalt kaum nach, obgleich er unter dem Kanadischen — sogar unter dem dortigen weichen Weizen der Küstenprovinzen — liegt.

Die Müller bevorzugen im allgemeinen Weizen mit hohem Stickstoffgehalt, und deshalb sind die in den Prärieprovinzen in Kanada angebauten Weizen am meisten gefragt. Der Stickstoffgehalt ist aber bei weitem

¹⁾ F. T. Shutt, Trans. Roy. Soc. Canada, 1935, Bd. 29, S. 1.

²⁾ Bei Weizen ist es üblich, hauptsächlich in Kanada, den Prozentgehalt an Protein anzugeben = $N \times 5,7$. Der so gewonnene Proteingehalt beträgt für englische Weizen 11,4 %; aber da er gewöhnlich auf 15 % Feuchtigkeit bezogen ist, vermindert er sich auf 9,7 %. Für die obigen kanadischen Durchschnitte ergeben sich die Werte von 16,5 und 13,5.

nicht der einzige Wertmesser für das Getreide; es wurden auch Mahl- und Backversuche durchgeführt, bei welchen die Weizen von Rothamsted nicht gut abgeschnitten haben. Die entsprechenden Versuche mit Weizen aus Woburn unterblieben.

Die Ursachen für die Schwankung im Stickstoffgehalt scheinen für Weizen und Gerste völlig die gleichen zu sein, sie äußern sich nur bei den Getreidearten verschieden. Bei beiden Arten erhöhte sich der Stickstoffgehalt im Korn durch Natronsalpeter; zusätzliche Gaben von Mineralsalzen verringerten ihn. Die Jahre mit hohem Stickstoffgehalt bei Gerste zeichnen sich gewöhnlich auch durch einen Stickstoffreichtum bei Weizen aus; aber die Zahlen für beide Getreidearten stehen nicht im direkten Zusammenhang. Wie bei Gerste wird auch bei Weizen der Stickstoffgehalt in Jahren mit hohen Erträgen niedriger und umgekehrt. Selbst Niederschläge beeinflussen die beiden Getreidearten in derselben Richtung; allerdings ist das Ausmaß der Wirkung verschieden. Erhöhte Niederschläge im März pflegen den Stickstoff zu steigern, vermindern ihn aber, wenn sie während der Hauptwachstumszeit (Mai bis Juni) auftreten. Erst zur Erntezeit reagieren die beiden Getreidesorten verschieden. Erhöhte Niederschläge im August bewirken eine deutliche Steigerung des Stickstoffgehaltes bei Weizen, während man bei Gerste einen derartigen Einfluß nicht beobachten kann. Höhere Juli-Temperaturen verursachen zwar bei Gerste eine Vermehrung des Stickstoffgehaltes, aber nicht bei Weizen.

Hinsichtlich der Wachstumsperiode erhält man bei Weizen (bei Gerste ebenfalls) hier dasselbe Ergebnis wie an anderen Stationen. Bei einem Versuch mit kanadischen Weizen, der sich über 28 Jahre erstreckte, fand Shutt¹⁾, daß hoher Stickstoffgehalt mit trockenen, niedriger mit feuchten Bodenbedingungen in Zusammenhang stand. Aber wir haben von keiner anderen Stelle gehört, daß erhöhte Niederschläge im frühen August den Stickstoffgehalt gesteigert haben.

Für das auffallende Sinken des Stickstoffgehaltes bei Weizen nach 1906 um annähernd 0,2% kann keine Erklärung gefunden werden. Bei Gerste tritt ein solches Abfallen nicht ein.

Tabelle 51.
Stickstoffgehalt des Weizenkornes.

Parzelle	1882 bis 1886	1887 bis 1891	1892 bis 1896	1897 bis 1901	1902 bis 1906	1907 bis 1911	1912 bis 1916	1917 bis 1921	1922 bis 1926	Durch- schnitt 1882 bis 1926
1 und 7	2,14 ⊕	2,06	1,95	2,09	1,90	1,83	1,96	1,92	1,76	1,95
4	2,18 ⊕	2,11	1,90 ⊕	2,21	1,98	1,87	1,97	1,95	1,86 ⊕	2,01
3 a*)	2,42 ⊕	2,02	2,11	2,33	2,16	1,87	2,18	1,96	1,71 ⊕	2,08
6	2,18 ⊕	2,02	2,21	2,28	2,14 ⊕	1,78	2,12	1,92 ⊕	1,84	2,04
11b	2,29 ⊕	2,13 ⊕	2,05	2,17	2,08 ⊕	1,99	2,02	1,94	1,92	2,05

¹⁾ F. T. Shutt. Die Einflüsse der Witterung auf den Stickstoffgehalt des Weizens Trans. Roy. Soc. Canada. Vol. 21, p. 1.

*) 3 b von 1907 bis 1926.

⊕ bezeichnet den Durchschnitt von weniger als fünf Jahren.

1000-Korngewicht bei Gerste.

Gerste in Woburn hat im allgemeinen eher ein niedrigeres 1000-Korngewicht, als es Gersten mit guter Malzqualität sonst besitzen; es bewegt sich zwischen 28,7 und 49,1 g mit einem Mittelwert von 37,2 g; bei guten Braugersten liegt es gewöhnlich zwischen 38 und 40 g.

Die Düngung hat hierbei keinen wesentlichen Einfluß: ohne Rücksicht auf die Düngung sind alle Werte in jedem einzelnen Jahr praktisch gleich.

Stark ausgeprägt ist indessen der Jahreseinfluß, wie die von Jahr zu Jahr beträchtlich abweichenden Werte beweisen; aber es ist sehr auffallend, daß keiner der meteorologischen Faktoren auf die Kornbildungs- oder -reifezeit besonderen Einfluß ausübt. Zwischen Niederschlägen, Sonnenschein-Stunden und Temperaturen im Mai, Juni, Juli oder August konnte irgendwelcher Zusammenhang nicht festgestellt werden. Die einzigen Beziehungen, die nachgewiesen werden konnten, lagen in Monaten, die zeitlich der Vegetationsperiode so fern standen, daß ein erklärlicher Einfluß nicht vorliegt. So senkten Niederschläge im Januar, Februar und März das 1000-Korngewicht, Februar-Regen hatte den nachhaltigsten Einfluß und machte 19 % der jährlichen Abweichung aus: selbst Niederschläge zwischen September und November schienen noch eine gewisse Wirkung auszuüben. Überdurchschnittliche Temperaturen im März und April steigerten im allgemeinen das 1000-Korngewicht und sind für etwa 17 % der Jahreschwankungen verantwortlich zu machen. Sonnenschein aber hatte in keinem Monat irgendwelchen nennenswerten Einfluß, eine Tatsache, die ganz im Gegensatz steht zu der allgemeinen Behauptung, daß Sonnenschein im Juni die Kornbildung fördert.

Es bestand also kein Zusammenhang zwischen 1000-Korngewicht einerseits und Stickstoffgehalt des Korns oder Verhältnis von Korn zu Stroh andererseits.

Tabelle 52.

Durchschnittsgewicht für Weizen und Gerste in Doppelzentner je Hektar.
1877 bis 1926.

Ungedüngt (Parzelle 1 und 7)	23,1
Nur Mineralsalze (Parzelle 4)	23,3
Parzellen	Parzellen
mit Natronsalpeter	mit schwefelsaurem Ammoniak
Nur Natronsalpeter (Parzelle 3 a)	Nur schwefelsaures Ammoniak (Parzelle 2 a)
Natronsalpeter + Mineralsalze (Parzelle 6)	Schwefelsaures Ammoniak + Mineralsalze (Parzelle 5 a)
Doppelter Stickstoff (Parzelle 9)	Doppelter Stickstoff (Parzelle 8)
Natronsalpeter fortgelassen (Parzelle 9b)	

2. Die durch ständigen Getreidebau bedingten Bodenverschlechterungen.

Mehrfacher Getreideanbau auf demselben Land.

Die heutigen Verhältnisse machen es oft erforderlich, von den festen Fruchtfolgen Abstand zu nehmen und häufig sind Befürchtungen laut geworden, daß diese Maßnahmen dem Boden schädlich seien. Der Anbau

von Getreide mehr als zweimal nacheinander auf demselben Land gilt in Großbritannien allgemein als schlechte Feldbewirtschaftung. Da heute durch das Anteil-System (Kontingente) der Anbau von Weizen in England gefördert wird, ist die Frage oft erörtert worden, wie weit sich unter den heutigen Verhältnissen ein Daueranbau von Feldfrüchten durchführen läßt.

Für die Beantwortung dieser Frage bietet der Getreideanbau sowohl in Woburn als auch in Rothamsted ein gutes Beispiel. Ohne irgendwelche Gefahr der Ertragsdrückung oder der nachteiligen Einwirkung auf den Boden kann jahrelang nacheinander auf demselben Land erfolgreich Getreideanbau durchgeführt werden; Voraussetzung dafür ist allerdings, daß das Unkraut auf das Mindestmaß beschränkt werden kann und daß im Boden lebende Erreger von Pflanzenkrankheiten wie Getreide-Älchen, Fußkrankheit (*Fusarium*), „Schwarzbeinigkeit“ (*Ophiobolus*) usw. nicht überhand nehmen. Auf den drei für ständigen Getreideanbau am meisten bekannten Versuchsfeldern in Rothamsted, Woburn und Sawbridgeworth (Versuchswirtschaft des Herrn Prout) konnte keine dieser Krankheiten beobachtet werden. Bereits nach nur wenigen Jahren ständigen Weizenbaus hatten auf den Kreideböden in Norfolk und den südlichen Provinzen die Farmer, die zur Mechanisation übergegangen waren, schwere Bodenschädigungen durch *Ophiobolus* und *Fusarium* zu verzeichnen. Wodurch wurde dieser Unterschied verursacht? Eine sichere Erklärung dafür kann noch nicht abgegeben werden, der Unterschied wird augenscheinlich durch die Menge der im Boden vorhandenen Kreide bedingt sein. Dieser vermehrte Kreidgehalt scheint das Auftreten von Fußkrankheiten und „Schwarzbeinigkeit“ zu begünstigen; weder in Rothamsted noch in Sawbridgeworth konnte man eine wesentliche und in Woburn überhaupt keine Steigerung des Kreidgehaltes feststellen.

Es wäre eine dankenswerte Aufgabe, über dieses Krankheitsproblem noch weitere Untersuchungen durchzuführen; aber bereits heute dürfen wir feststellen, daß man Getreide unbedenklich mehrere Jahre nacheinander auf demselben Land anbauen kann, wenn keine Krankheiten vorliegen, die geeignete Düngung gegeben wird und genügende Maßnahmen zur Bekämpfung des Unkrauts getroffen werden. Allerdings wird nach langer Zeit eine Verschlechterung doch nicht ausbleiben.

Gegenwirkung von künstlichen Düngemitteln und Stallmistdüngung.

Tabelle 31 zeigt, daß die Erträge in den ersten Jahren auf allen Parzellen steigen, aber dann fallen; allerdings macht sich das Absinken auf einigen Parzellen weniger bemerkbar als auf anderen. Nach 1906, als die Düngung geändert worden war, wurde das Abfallen stärker; wir können aber nicht beurteilen, ob diese Tatsache auf die verminderte Düngung zurückzuführen ist oder auf irgend einen nachteiligen Faktor, der von Jahr zu Jahr mehr an Bedeutung gewann. Beide ungedüngte Parzellen zeigten ein weiteres Sinken der Erträge, auch bei Parzelle 4

war dies der Fall, bei welcher die Änderung in der Düngung wahrscheinlich keine große Wirkung gehabt hätte. Diese Ertragsverschlechterung ist nicht den besonderen Bodenverhältnissen in Woburn zuzuschreiben, denn man findet sie auch auf dem völlig anderen Boden in Rothamsted. Obgleich wegen des Unterschiedes in der Versuchsdauer ein genauer Vergleich nicht möglich ist, läßt sich doch im allgemeinen eine Übereinstimmung der Ergebnisse beobachten.

Es ist schwer festzustellen, weshalb Getreide, das künstliche Vollendung erhalten hat, beim jahrelangen Anbau auf demselben Land im

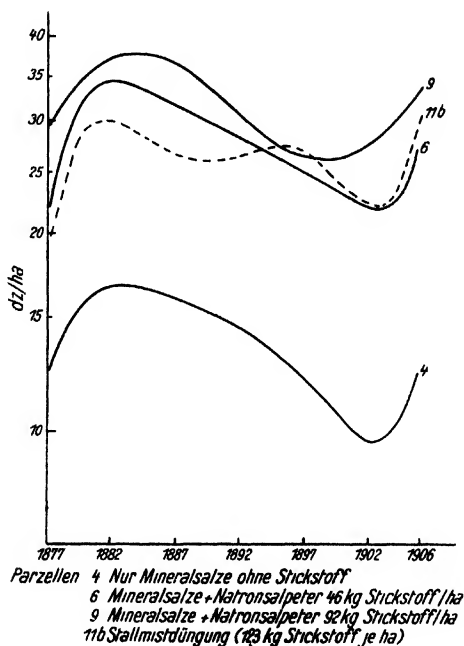


Abb. 10.
Kornsertrag bei Gerste.

Erträge nachlassen sollte. Die Verschlechterung ist nicht allein die Folge eines Anbaus ohne Fruchtwechsel; sie findet sich in Rothamsted und in Woburn auch auf den Parzellen mit Fruchtwechsel.

Die nach 30 Jahren vorgenommene Änderung in der Düngung, die einen Vergleichsversuch für die Ergebnisse in Woburn unmöglich machte, erbrachte nicht den geringsten Vorteil, sondern beeinträchtigte den Wert der Arbeit außerordentlich. Eine Lehre kann man jedenfalls aus den Versuchen in Woburn ziehen: Wenn einmal ein Versuch auf lange Sicht begonnen ist, soll man zwischendurch den Versuchsplan nicht ändern.

Den vollständigen Bericht darüber bringt die Monographie von Rothamsted „50 Jahre Feldversuche in Woburn“. Einige Kurven aus dieser Arbeit bringt Abb. 10. Diese unterscheiden sich von der sonst

üblichen Form dadurch, daß die Zahlen den Logarithmus der Erträge und nicht die Erträge selbst darstellen. Diese Methode hat den Vorteil, daß sie einen Vergleich der einzelnen Behandlungsarten gestattet. Der gleichmäßige Verlauf dieser Kurven gibt die durch die Verschlechterung hervorgerufenen gleichmäßigen prozentischen Veränderungen der Erträge an.

Alle Kurven zeigen übereinstimmend am Anfang der Versuchszeit ein starkes und am Schluß ein leichtes Ansteigen. Welche Sicherheit man diesen Veränderungen beimessen kann, läßt sich deshalb nicht sagen, weil die Anfangs- und die Endzahlen in den aufgestellten Serien nicht so zuverlässig sein können wie die übrigen. Die allgemeine Richtung ist wahrscheinlich maßgebend, wenn auch die tatsächlichen Werte unsicher sein mögen.

Die Tatsache, daß man bei allen Kurven zu Versuchsbeginn ein Ansteigen beobachten konnte, weist entweder darauf hin, daß in den allerersten Jahren die Witterungsverhältnisse ständig sehr günstig waren oder daß das Land zu Versuchsbeginn besser bearbeitet wurde. Da das Ansteigen sich sowohl auf den ungedüngten wie auch auf den anderen Parzellen bemerkbar machte, kann man es natürlich nicht einer zunehmenden Wirksamkeit der Düngemittel zuschreiben.

Die Grade der Verschlechterung in dem Zeitraum von 1877 bis 1906 sind:

	Parzelle	Weizen		Gerste	
		Doppel- zentner je Hektar jährlich	%	Doppel- zentner je Hektar jährlich	%
Natronsalpeter . . .	3a	0,334	1,56	0,703	2,25
Ungedüngt	1	0,410	3,22	0,701	3,79
Ungedüngt.	7	0,286	2,01	0,425	2,34

Das auffallendste Ergebnis ist das, daß nur eine Düngungsart die Erträge ständig auf der gleichen Höhe hielt, nämlich Stallmistdüngung, die in den ersten 30 Jahren in einer Gabe von ungefähr 200 dz, in den letzten 20 Jahren in einer Menge von etwa 125 bis 150 dz je Hektar im Jahr zur Anwendung kam.

Keine andere Düngungsmethode erhielt die Erträge so hoch. Künstliche Volldüngung mit Natronsalpeter als Stickstoffquelle kam der Stallmistdüngung am nächsten, bei Weizen blieb sie nicht weit hinter ihr zurück, bei Gerste dagegen zeigte sie sich weniger wirksam. Bei stärkeren Dosen von Natronsalpeter ähnelt die Kurve für künstliche Düngemittel mehr derjenigen des Stallmistes. Gleiche Stickstoffgaben, z. B. 123 kg je Hektar Stickstoff als Natronsalpeter, können möglicherweise dazu führen, daß der Unterschied, der in der Natur der organischen Substanz begründet liegt, vielleicht sehr gering wird. Diese Möglichkeit dürfte von einigem Interesse sein, weil der prozentische Kohlen- und Stickstoffgehalt des Bodens auf Parzelle 9 abgenommen hat, ganz im Gegensatz zu der Stall-

mistparzelle. Das beweist nicht den oft vertretenen Standpunkt, daß Natronsalpeter bei alleiniger Anwendung den Boden besonders stark zu erschöpfen pflegt, obgleich diese Düngung natürlich ohne Kali und Superphosphat weniger wirksam ist, als wenn die genannten Düngemittel zusätzlich gegeben werden. Trotz des höheren Ertrags und der größeren Beanspruchung des Bodens in bezug auf Mineralsubstanzen ist die Verschlechterung auf den nur mit Natronsalpeter gedüngten Parzellen nicht schneller fortgeschritten als auf den ungedüngten.

Von den beiden Gruppen der in den Düngemitteln wirksamen Bestandteile ist für die Erhaltung der Weizenenerträge der Stickstoff von größerer Bedeutung, während für die Erhaltung der Gerstenenerträge die mineralischen Düngemittel von Wichtigkeit sind (Abb. 10).

Dieser Unterschied zwischen Weizen und Gerste führt noch zu einigen weiteren auffallenden Ergebnissen. Der Stickstoff behielt seine Wirksamkeit gegenüber Weizen bei, steigerte Gesamtertrag und Kornenertrag und hielt diese bis zum Abschluß des Versuches sehr gut auf der gleichen Höhe. Aber für Gerste ließ die Wirksamkeit des Stickstoffs nach, hier wurde die Steigerung in den späteren Jahren geringer. (Tabelle 53). So brachte beim Versuchsbeginn 46 kg Stickstoff, der als Natronsalpeter zu der künstlichen Volldüngung zusätzlich gegeben wurde, eine Steigerung des Gesamtertrages um etwa 82 kg bei Gerste und 78 kg bei Weizen; in den letzten 20 Jahren betrug die Zunahme für Weizen 73 kg, für Gerste allerdings nur etwa 40 kg. Dagegen fiel der Ertrag bei Weizenparzellen ohne Stickstoffgabe stärker als bei denjenigen, die Stickstoff erhalten hatten, so daß die prozentische Zunahme stieg. Die Steigerung machte sich auf Parzelle 9 mit der doppelten Stickstoffgabe stärker be-

Tabelle 53.
Steigerung durch Natronsalpeter und Stallmistdüngung.

	Die ersten 15 Jahre		Die zweiten 15 Jahre		Die letzten 20 Jahre	
	Gerste	Weizen	Gerste	Weizen	Gerste	Weizen
Kornenertrag je Kilogramm zugeführten Stickstoffs.						
Stallmistdüngung	8	6	10	8	11	8
Natronsalpeter	29	24	24	22	18 ¹⁾	25
Wirksamkeit des Stickstoffes in Stallmistdüngung (N in Natronsalpeter = 100)					15 ²⁾	
	28	24	42	36	67	32
Gesamtertrag je Kilogramm zugeführten Stickstoffs.						
Stallmistdüngung	17	17	24	23	26	24
Natronsalpeter	73	70	59	58	42	65
Wirksamkeit des Stickstoffes in Stallmistdüngung, (N in Natronsalpeter = 100)						
	23	24	41	40	62	57

¹⁾ Parzelle 6, aber mit halber Düngung.

²⁾ Parzelle 9, aber dieselbe Düngung wie vorher.

merkbar als auf Parzelle 6 mit der einfachen. Als nach 1906 die Stickstoffdüngung halbiert wurde, wurde die prozentuale Zunahme gleichfalls geringer.

Bei Gerste erhöhte sich die prozentuale Zunahme fast in demselben Maße wie bei Weizen auf den Parzellen ohne Mineralsalze, kam diese Düngung aber zur Anwendung, so ließ das Steigen der prozentualen Zunahme beträchtlich nach. Aus dieser relativen Beständigkeit kann man schließen, daß sich die Wirksamkeit des in der Stallmistdüngung enthaltenen Stickstoffs — wenn man den im Natronsalpeter vorhandenen = 100 setzt — bei Gerste während der ganzen Versuchsdauer, bei Weizen bis zu einem bestimmten Zeitpunkt erhöht (Tabelle 53).

Im engen Zusammenhang mit der Verschlechterung des Ertrages — und wahrscheinlich als unmittelbare Ursache dafür — steht eine verminderte Stickstoffaufnahme der Pflanze. Mit dieser Folge hatte man nur bei den Parzellen ohne Stickstoff gerechnet. In den ersten Jahren nahm die Gerste annähernd 32 kg je Hektar Stickstoff aus dem Boden auf, in den letzten Jahren nur 9 kg je Hektar aus der ungedüngten und 13 kg je Hektar aus der nur mit Mineralsalzen gedüngten Parzelle. Hieraus könnte geschlossen werden, daß der wirksame Stickstoff verbraucht war; aber ein ähnliches, allerdings entsprechend geringeres Abfallen kam auch bei den jährlich mit Natronsalpeter gedüngten Parzellen vor.

In den 15 Jahren, von 1877 bis 1891, war die Ertragsdrückung nur gering; die Gerste auf Parzelle 6 nahm ungefähr 69 kg Stickstoff je Hektar jährlich auf, das heißt 38 kg mehr als auf Parzelle 4 ohne Natronsalpeter, was einer Ausnutzung von etwa 90 % gleichkommt. Als sich die Verschlechterung in den letzten fünf Jahren, 1922 bis 1926, deutlich bemerkbar machte, betrug die Stickstoffaufnahme der Gerste durchschnittlich nur ungefähr 22 kg je Hektar, also annähernd 10 kg mehr als auf Parzelle 4, mit einer Ausnutzung von nur etwa 45 %.

Abgesehen von der Größe konnte man an den Pflanzen kaum eine Veränderung wahrnehmen. Im Vergleich zu den vergangenen Jahren war das Stroh natürlich kürzer geworden und das Verhältnis von Korn zu Stroh blieb gleich, bis es gegen den Schluß der 50 Jahre fiel; wie später gezeigt wird, war dieses Absinken nur scheinbar und nicht wirklich. Auch das Hektoliter-Gewicht des Korns und das 1000-Korngewicht bewegten sich während der ganzen Zeit ungefähr in der gleichen Höhe; sie zeigten keine eigentliche Tendenz zum Fallen, erst gegen Ende des Versuches setzt diese ein. Solche geringe Abweichungen traten bei Gerste stärker auf als bei Weizen. Der Stickstoffgehalt der Gerste pflegte stabil zu sein, Schwankungen nach oben oder unten traten nicht auf.

Wie zu Beginn so zeigte auch gegen Abschluß des Versuches der von den Pflanzen aufgenommene Stickstoff dieselbe wachstumsfördernde Wirkung. Sowohl in den ersten wie in den letzten Versuchsjahren entsprach jedes Kilogramm aufgenommenen Stickstoffs ungefähr 100 dz des Gesamtertrages.

Im Laufe des Versuchs waren wiederholt Bodenproben den verschiedenen Parzellen entnommen worden und diese sind in Rothamsted unter Dr. Crowther's Leitung untersucht worden. Ein Einzelversuch über Kohlen- und Stickstoffgehalt der Böden ist 1933 von A. Walkley durchgeführt worden. Beim Versuchsbeginn 1876 war der Gehalt an Kohlenstoff und Stickstoff gleich hoch; im Verlauf des Versuches fiel er, nur die Stallmistparzelle wurde davon nicht betroffen. Die Zahlen hierfür, ausgedrückt in Prozenten des lufttrockenen Bodens, sind etwa folgende:

	Zu Beginn des Ver- suches 1876	Beim Abschluß des Versuches 1926	
		Parzelle mit Stallmistdüngung	Ungedüngte und nur mit künst- lichen Mitteln gedüngte Parzelle
Kohlenstoff . . .	1,48	1,5	1,0
Stickstoff . . .	0,155	0,15	0,09

Die verschiedenen künstlichen Düngungsgaben verhielten sich annähernd gleich. Auch zwischen Weizen- und Gerstenparzellen bestanden kaum Unterschiede; falls diese vorhanden waren, sind sie nicht auf die Frucht oder auf die Düngung zurückzuführen, sondern können unbedenklich den Ungleichmäßigkeiten im Boden zugeschrieben werden. Die Zahlen für Kohlenstoff sind nicht als unbedingt zuverlässig anzusehen, weil auf einigen Parzellen Restteile von Kohle gefunden worden sind, deren Herkunft und Menge sich nicht feststellen läßt. Die in Tabelle 16 niedergelegten Zahlen für Stickstoff gewähren eine größere Sicherheit und lassen Schlußfolgerungen hinsichtlich der Veränderungen in der Menge der organischen Substanz zu.

Andererseits sind diese Zahlen kein Beweis dafür, daß sowohl hinsichtlich des Versuchsverlaufes als auch hinsichtlich der einzelnen Parzellen untereinander kein Unterschied in der Natur der organischen Bodensubstanz besteht. Das Verhältnis Kohlenstoff- zu Stickstoffgehalt des Bodens bleibt auf ungefähr 9,7; zwischen der ungedüngten und der Stallmistparzelle einerseits und den ersten und letzten Versuchsjahren andererseits konnte nicht der geringste Unterschied festgestellt werden¹⁾.

Das Nachlassen in der Menge der organischen Bodensubstanz zeigt offenbar Ähnlichkeiten mit der Verschlechterung der Erträge, obgleich hierfür keine enge Beziehung besteht. So ist z. B. keine Beziehung vorhanden zwischen Kohlen- oder Stickstoffgehalt der Böden am Schluß der Versuchsperiode und den durchschnittlichen Erträgen in den vorhergehenden zehn Jahren.

Innerhalb der 50jährigen Versuchszeit verminderten sich zwar auch die im Boden ersetzbaren Basen; aber diese Veränderungen können, wie zu erwarten war, ganz eindeutig auf die Düngung zurückgeführt werden.

¹⁾ Ein höheres Verhältnis zeigten einige Weizenparzellen; aber es waren diejenigen, bei welchen Kohleteichen gefunden worden waren.

Tabelle 54.
Ersetzbares Kalzium in Milligramm gleichbedeutend mit 100 g Boden
zu verschiedenen Zeiten.

	Par- zelle	Gerste-Parzellen			Weizen-Parzellen		
		1888	1898	1927	1888	1898	1927
Ungedüngt	1	6,85	6,72	3,76	7,08	6,96	4,76
Ungedüngt	7	7,20	—	4,03	7,71	—	4,08
Nur Mineralsalze	4	5,53	5,13	4,96	7,16	6,01	6,23
Schwefelsaures Ammoniak + Mineral- salze	5a	5,44	3,65	1,56	5,47	4,36	1,82
Natronsalpeter + Mineralsalze	6	7,12	—	5,46	6,81	—	5,24
Stallmistdüngung	11b	7,67	—	6,44	8,41	—	6,06

Die Ergebnisse für Kalzium (den bei weitem wichtigsten Bestandteil) in der Bodenoberfläche (23 cm) bringt Tabelle 54.

Man konnte hier auf allen Parzellen ein Abfallen beobachten, das in einer gewissen Beziehung zu der Verschlechterung steht. Am deutlichsten tritt es bei den sauren Parzellen auf, man findet es auch, wenn auch nicht so ausgeprägt, auf den Parzellen ohne Stickstoffdüngung; das Sinken ist am geringsten auf den Parzellen, die Stallmistdüngung oder künstliche Volldüngung mit Natronsalpeter als Stickstoffquelle erhalten haben. Vollständige Angaben über die anderen Basen fehlen; diese sind auch nur in geringen Mengen vorhanden. Magnesium ist noch von einer gewissen Bedeutung, aber soweit Ergebnisse dafür vorliegen, bewegen sich die Veränderungen in der gleichen Richtung wie bei Kalzium.

Wirkung der Brache.

Welche Ursache die Verschlechterung auch haben mag, sie kann für eine gewisse Zeit durch eine Voll-Brache aufgehoben werden. Nach Ab-

Tabelle 55.
Einfluß der Brache hinsichtlich der Wiederherstellung
der Produktivität nach dauerndem Anbau.

	Un- gedüngt	Nur Mineral- salze	Nur Natron- salpeter	Natron- salpeter + Mineral- salze	Stallmist- düngung
	Parzelle 1 und 7	Parzelle 4	Parzelle 3a und b	Parzelle 9 ¹⁾	Parzelle 11a
Die letzten 5 Jahre des dauernden Anbaus, 1922 bis 1926.	3,4	4,9	5,3	6,9	13,8
Nach einer zweijährigen Brache (bis 1928)	11,8	12,3	18,2	21,6	20,2
Die ersten fünf Jahre 1877 bis 1881. Verschlechterung nicht festzu- stellen	13,7	12,6	20,0	20,8	21,1

¹⁾ E. M. Crowther und J. K. Basu, Journ. Agr. Sci. Vol 21, p. 639 Die landwirtschaftliche Bedeutung dieser Veränderungen wird von E. M. Crowther behandelt in Journal Roy. Agr. Soc. 1932, Vol. 93.

schluß der 50 jährigen Versuchszeit blieben die Parzellen für die beiden Jahre 1927 und 1928 brach. 1929 wurden sie wieder bestellt, aber nicht gedüngt.

Die Wirkung dieser zweijährigen Brache war deutlich zu bemerken. Auf den meisten Parzellen stiegen die Erträge bis zu der Höhe der ersten fünf Jahre (1876 bis 1881), als sich noch keine Anzeichen der Verschlechterung bemerkbar gemacht hatten. Nur Parzellen mit schwefelsaurem Ammoniak, die sauer geworden waren, wichen ab; auf Säure hat die Brache augenscheinlich wenig Einfluß. Die Kornerträge für Gerste in Doppelzentner je Hektar bringt Tabelle 55 (und auch Tabelle 48).

Der Haupterfolg der Brache dauert nur ein Jahr; danach sinken die Erträge beträchtlich.

Wirkung des Fruchtwechsels.

Die Verschlechterung ist nicht allein auf das Land mit Daueranbau beschränkt, sie tritt auch auf dem Land mit Fruchtwechsel auf. Auf dem anderen Teil des Stackyard-Feldes ist ein Fruchtwechselversuch durchgeführt, der sowohl Weizen als auch Gerste umfaßt. Die in Tabelle 56 zusammengefaßten Ergebnisse zeigen verschiedentlich ein stärkeres Abfallen als auf den Parzellen mit ständigem Weizen- oder Gerstenbau. Ein Vergleich zwischen Daueranbau und Fruchtwechsel läßt sich im einzelnen

Tabelle 56.

Vergleich der Weizen- und Gerstenerträge bei Daueranbau und Fruchtwechsel auf dem Stackyard-Feld in Woburn.

Perioden	Ungedüngt	Natronsalpeter				Organische Düngung ¹⁾			
		Kornertrag Doppel- zentner je Hektar Mittlere Erträge		Stickstoff in der Düngung Kilogramm je Hektar jährlich		Kornertrag Doppel- zentner je Hektar Mittlere Erträge		Stickstoff in der Düngung Kilogramm je Hektar jährlich	
		Daueranbau	Fruchtwechsel	Daueranbau	Fruchtwechsel	Daueranbau	Fruchtwechsel	Daueranbau	Fruchtwechsel
Gerste									
1878 bis 1885	15,1	28,6	30,4	47	—	25,2	28,9	123	—
1886 bis 1897	9,5	23,9	25,0	47	26	22,5	23,0	123	26
1905 bis 1910	7,2	17,3	25,5	24	52	22,8	29,3	92 ²⁾	52
1911 bis 1926	4,9	10,4	—	24	48	18,0	15,1	92	48
Weizen									
1878 bis 1885	10,8	21,2	28,6	47	44	17,7	28,4	123	44
1886 bis 1897	8,5	18,8	24,0	47	—	17,7	24,1	123	—
1905 bis 1910	7,8	15,3	14,2	24	—	15,7	13,8	92 ²⁾	—
1911 bis 1926	5,4	12,6	—	24	—	14,4	16,3	92	—

¹⁾ Stallmist bei dauerndem Gerstenbau, Schafmist bei Fruchtwechsel.

²⁾ 123 bis 1905/1906, danach 92.

nicht durchführen, weil die Düngung verschieden gewesen ist; die Parzellen mit Fruchtwechsel haben viel weniger Dünger erhalten als Weizen- oder Gerstenparzellen im Daueranbau.

Bei diesem Vergleich sind nur die Jahre herangezogen, in welchen Gerste beim Fruchtwechselversuch angebaut worden war. Während jeder der beiden ersten Zeiträume war Gerste siebenmal, innerhalb der letzten Periode sechsmal angebaut worden.

Bei der Berechnung der durchschnittlichen Werte ist der Weizen im Jahre 1931 nicht berücksichtigt worden, weil er auf dem Agdell-Feld durch mangelnde Keimfähigkeit oder Frostscha den kaum gekommen war.

Die Ergebnisse von Woburn lehren weiter, daß die Verschlechterung durch kleine Gaben organischer Substanzen nicht aufgehoben werden kann. Eine Zeitlang erhielten die im Fruchtwechsel stehenden Parzellen alle vier Jahre eine geringe Menge Stallmist (75 bis 100 dz je Hektar) und zwei Jahre später wurden auf den Rübenparzellen Schafe gepercht, die mit kleinen Mengen Kuchen oder Korn gefüttert wurden. Leider ist der Düngungsplan so viel geändert worden, daß man über die Wirkungen der einzelnen Düngemittel kein sicheres Urteil abgeben kann; jedenfalls war die Gesamtwirkung der Düngungen so unzulänglich, daß die Verschlechterung nicht aufgehoben werden konnte. Auf den Gründungsparzellen wurde jedes Jahr Senf oder Wicke eingepflügt, aber auch hier nahm die Verschlechterung genau so schnell zu wie auf den ungedüngten Parzellen.

Auf dem Agdell-Feld in Rothamsted, wo seit 1848 im viermaligen Fruchtwechsel Rüben, Gerste, Klee und Weizen angebaut war, zeigte sich bei Klee-Rückständen ein ähnlicher Nachteil insofern, als auch diese die Verschlechterung nicht verhüten konnten (Tabelle 57). Stallmistdüngung hatten diese Parzellen nicht erhalten.

Tabelle 57

Weizen- und Gerstenerträge bei Daueranbau und bei Fruchtwechsel.
(Durchschnitt von drei Zeiträumen) Rothamsted.

Korntrag in Doppelzentner je Hektar.

Jahre	Weizen				Jahre	Gerste			
	Ungedüngt		Gedüngt			Ungedüngt		Gedüngt	
	Daueranbau Broadbalk 3	Fruchtwechsel Agdell 5 und 6	Daueranbau Broadbalk 6	Fruchtwechsel Agdell 1 und 2		Daueranbau Hoos 1—0	Fruchtwechsel Agdell 5 und 6	Daueranbau Hoos 4—A	Fruchtwechsel Agdell 1 und 2
1851 bis 1875	7,9	16,7	14,8	19,1	1853 bis 1877	11,0	17,7	28,6	26,4
1897 bis 1908	6,4	11,3	12,1	20,0	1881 bis 1905	5,2	9,9	19,3	17,3
1907 bis 1927	5,2	8,9	10,3	11,8	1909 bis 1929	6,0	6,4	18,7	11,6

Man darf nicht annehmen, daß der Fruchtwechsel ohne Einfluß auf die Bodenfruchtbarkeit blieb. Im Gegenteil, aus der Tabelle ergibt sich,

daß die Erträge der in Fruchtwechsel stehenden Parzellen mit sehr kleinen Stickstoffgaben fast die gleiche Höhe erreichen wie die Erträge der Parzellen im Daueranbau, die bedeutend mehr Stickstoff erhalten haben. Aber wie gerade die stärkeren Düngegaben bei Daueranbau eine Verschlechterung nicht verhindern können, ebensowenig kann das der Fruchtwechsel allein. Zweifellos würde die Verwendung von Stallmist beim Fruchtwechselversuch den Eintritt einer Verschlechterung vermeiden, wie das in der Praxis tatsächlich der Fall ist. Es wäre interessant, festzustellen, welche Düngermenge auf den Parzellen mit Fruchtwechsel einer Düngergabe von 200 dz Stallmist auf den Parzellen mit Daueranbau entsprechen würde

Die Ausführungen über die Verschlechterung lassen sich in folgende Punkte zusammenfassen:

1. Im Laufe der Jahre fällt die von den Pflanzen aus dem Boden aufgenommene Stickstoffmenge sehr beträchtlich und in dem gleichen Maße läßt auch das Wachstum der Pflanzen nach.
2. Die Ertragsdrückung ist auf die in den Pflanzen vor sich gehenden Prozesse ohne Einfluß. Sie verändert weder die Wirkung des zur Bildung von Pflanzensubstanz aufgenommenen Stickstoffs noch den zur Kornbildung in der Pflanze weiterwandernden Anteil an Stickstoff oder den prozentischen Stickstoffgehalt im Korn oder das „bushel“-Gewicht des Korns. Dieses kann allerdings abnehmen, wenn die Verschlechterung sehr stark wird.
3. Keine Verschlechterung oder nur eine geringe zeigt sich bei Parzellen, die ungefähr 200 dz Stallmist (etwa 123 kg Stickstoff je Hektar) jährlich erhalten haben. Bei künstlicher Volldüngung nimmt die Verschlechterung bereits etwas zu; sie macht sich auf den unvollständig oder nicht gedüngten Parzellen stärker bemerkbar und tritt auf den sauren Parzellen am deutlichsten in Erscheinung.
4. Die Verschlechterung bringt eine Abnahme im Gehalt an Kohlenstoff, Stickstoff, ersetzbarem Kalzium und anderen im Boden vorhandenen Basen mit sich; aber die Abnahme erfolgt nicht genau proportional der Verschlechterung. Bei der Stallmistparzelle konnte man weder eine Verschlechterung im Ertrag noch eine Abnahme an Stickstoff feststellen. Auf den Parzellen ohne künstliche Düngung konnte man sowohl eine Verschlechterung als auch einen Stickstoffverlust deutlich beobachten, bei den Parzellen mit künstlicher Volldüngung war die Stickstoffabnahme fast dieselbe, wenn auch nicht ganz so groß, aber die Ertragsdrückung war geringer. Andererseits entspricht bei den ungedüngten oder nur künstlich gedüngten Parzellen die Abnahme an ersetzbaren Basen dem Maße der Verschlechterung. Allerdings ist auf den Stallmistparzellen, die keine Verschlechterung aufweisen, der Verlust an Basen sehr groß.
5. Die Brache kann die Verschlechterung zeitweilig aufhalten, aber nach dem ersten Jahre fallen die Erträge schnell bis auf ihren alten Stand zurück.

6. Die Verschlechterung ist keine für den Daueranbau charakteristische Folgeerscheinung; sie tritt auch bei den in Fruchtwechsel stehenden Parzellen auf, wenn diese sogar Klee als Gründüngung, allerdings keinen Stallmist, erhalten haben.

Die durch Unkraut hervorgerufenen Veränderungen erschweren eine Erklärung. Sobald das Absinken der Erträge einsetzt, vermehrt sich das Unkraut und seine Ausrottung ist mit den größten Schwierigkeiten verknüpft. Es entzieht den Pflanzen oberhalb des Bodens das Licht und im Boden Feuchtigkeit und Nährstoffe und schädigt so die bereits kümmernden Pflanzen. Zur Ernte werden die Halme des Unkrauts mit erfaßt und mit dem Stroh gewogen, so daß dadurch das Verhältnis Korn zu Stroh unter seinen eigentlichen Wert fällt. Die Brachezeit hat eine Ausmerzung des Unkrautes zur Folge und bei der ersten Ernte nach der Brache kommt kein Unkraut vor; aber in den späteren Jahren mit den geringeren Erträgen tritt das Unkraut bald wieder in demselben Umfang wie früher auf.

Es durfte unmöglich sein, sämtliche Verschlechterungserscheinungen auf das Unkraut zurückzuführen. Man könnte daraus schließen, daß das Land niemals wieder den guten Zustand der Jahre 1876 bis 1891 erreicht hat, daß die Bearbeitung regelmäßig nachließ und in dem Zeitraum von 1922 bis 1926 am wenigsten erfolgreich war.

Eine andere Erklärungsmöglichkeit deutet auf die alte, vor 100 Jahren von Candolle aufgestellte Hypothese hin, daß die Absonderungen der Pflanzenwurzeln für die eigene Art schädlich, für andere Pflanzengattungen aber ohne Nachteil sind. Diese Ansicht ist nicht bewiesen; gegen diese Annahme spricht jedenfalls die Tatsache, daß die Verschlechterungen sowohl bei den Parzellen mit Fruchtwechsel als auch bei denjenigen mit Daueranbau auftreten.

Zur Erklärung der Verschlechterungen könnte weiterhin das verstärkte Auftreten von Krankheiten in Betracht gezogen werden, eine Tatsache, die für die Verhältnisse in Woburn vielleicht von Wichtigkeit ist. Eine Fußkrankheit (*Ophiobolus graminis*) verursacht dort große Verluste: leider läßt sich aus den Aufzeichnungen nicht entnehmen, wann die Krankheit zuerst auftrat. Aber für die Verschlechterung sind die Krankheiten im Grunde doch nur von untergeordneter Bedeutung, denn sie kommen für Rothamsted z. B. kaum in Frage.

Die wahrscheinlichste Ursache für die Verschlechterung scheint die Abnahme an solchen Bodensubstanzen zu sein, die unter Feldbedingungen für das richtige Wachstum der Pflanze notwendig sind. Die Tatsache, daß künstliche Düngemittel die Verschlechterung nicht vollständig aufhalten, zeigt, daß der fehlende Bestandteil weder Kali noch Phosphor ist; es kann sich auch nicht um Stickstoff handeln, da die Ertragssteigerung je Kilogramm Stickstoff mit fortschreitender Verschlechterung abnimmt. Es läßt sich sehr schwer entscheiden, ob die Verschlechterung hervorgerufen wird durch eine Erschöpfung an etwas weniger wichtigen Bestandteilen wie Bor oder Mangan, obgleich die von Hugh Ramage in Norwich

durchgeführte spektroskopische Prüfung der Pflanzenasche einen Mangel an diesen Substanzen nicht ergab; diese Tatsache kann aber nicht als Beweis gewertet werden. Wahrscheinlich steht die Verschlechterung mit der Abnahme der organischen Substanzen in Zusammenhang. Eine Erklärung hierfür ist nicht leicht. In Woburn sowohl als auch in Rothamsted war der Boden von guter physikalischer Beschaffenheit, und wenn die Wirkung der organischen Substanzen auf die Ernährung der Pflanze nur in einer Versorgung mit Stickstoff bestände, läßt sich kaum verstehen, warum künstliche Düngemittel nicht den gleichen Einfluß haben sollten.

Es ist klar, daß nicht alle Arten organischer Substanzen diese Wirkung haben. Weder Rapskuchen noch das Einpflügen von Senf oder Wicke oder der Mist eingepferchter Schafe oder Stallmist in geringen Mengen konnten eine Verschlechterung verhüten, nur Stallmist in verhältnismäßig hohen Gaben konnte dieses erreichen.

Beobachtungen lehrten, daß die wirksamen Bestandteile im Stroh vorhanden sein müssen. Versuche ergaben, daß unzersetztes Stroh allein die Bodenfruchtbarkeit nicht erhöht, daß sich aber während des Zersetzungsprozesses das Lignin des Strohs mit Protein verbindet und Humus ergibt. Es ist nicht bekannt, ob die Verschlechterung auf den Verlust an Humus oder irgendeinem anderen Zersetzungsprodukt des Strohs zurückzuführen ist. Der Verlust an Humus ist nur relativ, denn selbst auf den am meisten erschöpften Parzellen bleibt noch ungefähr 0,9 % Kohlen- und 0,09 % Stickstoff zurück. Es liegt überhaupt kein Grund zu der Annahme vor, daß dieser zurückgebliebene Humus sich gegenüber dem Pflanzenwachstum resistenter oder weniger wirksam erweist als der verbrauchte Humus. Die beiden sind chemisch aber nicht unterscheidbar. Wenn Humus der wirksame Bestandteil ist, können wir nur vermuten, daß bei der hier vorliegenden Höhe des Humusgehaltes die Bodenfruchtbarkeit je nach dem Prozentsatz des vorhandenen Humus steigt und fällt. Aber zurzeit kann man hier nur von einer Möglichkeit sprechen; eine Bestätigung ergeben erst weitere Versuche.

Selbst wenn der Beweis erbracht ist, daß Humus den wirksamen Bestandteil darstellt, bleibt immer noch zu erforschen, wie diese Wirkung vor sich geht.

Dieser Frage der Verschlechterung kam niemals ein praktischer Wert zu, solange es in der Praxis üblich war, Feldbau durchzuführen in vier- oder fünfjährigem Fruchtwechsel mit einer einmaligen guten Stallmistdüngung für diese Zeit, und Schafe auf Gras- und Rübenflächen zu pferchen. Auch für die Handelsgärtnerei spielte die Verschlechterung keine Rolle, solange die Gärtner Gelegenheit hatten, aus den Städten große Mengen Stallmist billig zu erhalten. Die Frage der Verschlechterung wurde für die Gärtner erst dann aktuell, als ihnen mit der Verdrängung der Pferde in der Stadt durch die Motoren ihre Zufuhr an Stallmist abgeschnitten wurde. An Bedeutung gewann diese Frage auch für die sogenannten „mechanisierten Bauern“, die ständig eine größere Fläche Land bebauen, als es ihrem Viehbestand entspricht.

Seit einigen Jahren laufen in Rothamsted Versuche, die jetzt ebenfalls in Woburn begonnen wurden, zur Klärung der Frage, ob Stroh außer als Stallmist auch noch in anderer Form dem Boden nutzbar zugeführt werden kann. Zwei Methoden sind genau geprüft worden: einmal läßt man das Stroh mit Hilfe von Mikroorganismen verrotten, diese Methode ist der allgemein bekannte Adco-Prozeß, der sich auf die in Rothamsted von Hutchinson und Richards durchgeführten Versuche aufbaut; bei der zweiten Versuchsanordnung pflügt man das Stroh ein und fügt gleichzeitig die für die Mikroorganismen notwendigen Nährstoffe in Gestalt einer Gabe künstlicher Düngemittel bei. Mit Stallmist ist ein Vergleichsversuch angestellt worden, dessen Resultate zu gegebener Zeit veröffentlicht werden sollen.

Die Unsicherheit der Gründungen.

Ein anderes Ergebnis, mit dem man bei den Versuchen in Woburn nicht gerechnet hatte, bestand darin, daß Gründungen für die Verbesserung der Weizenernte versagten. Beide Methoden, Senf und Wicke, wurden erprobt: sie hatten ein leichtes Ansteigen der organischen Substanz zur Folge und im Vergleich zu Senf enthielt Wicke im Durchschnitt 45 kg je Hektar mehr Stickstoff, der wahrscheinlich aus der Luft gewonnen war. Tabelle 58 bringt einige neue Ergebnisse.

Tabelle 58
Durchschnittlicher Ertrag in Doppelzentner je Hektar
nach eingepflügten Gründungen mit Senf und Wicke.

	Stackyard		Lansome		Beide Felder	
	Senf	Wicken	Senf	Wicken	Senf	Wicken
Frischgewicht						
1. Ernte	37,3 ¹⁾	102,7 ¹⁾	67,9	101,4	54,8	102,0
2. Ernte	12,6 ²⁾	6,5 ²⁾	20,9	74,7	21,4	40,7
Durchschnitt	27,4	64,3	55,3	92,4	42,6	79,6
Trockensubstanz.						
1. Ernte	7,5	17,8	16,2	24,1	11,8	21,0
2. Ernte	3,6	5,8	4,8	10,6	4,1	7,7
Durchschnitt	5,8	12,7	12,4	19,6	8,9	16,0
Stickstoff in Kilogramm je Hektar.						
1. Ernte	13	60	28	83	21	72
2. Ernte	11	25	12	72	11	44
Durchschnitt	13	45	22	78	18	62
Stackyard.			Lansome.			
1. Ernte	1930 bis 1933		1. Ernte	1902, 1905, 1930, 1932		
2. Ernte	1930 bis 1932		2. Ernte	1930, 1932		

¹⁾ Nur drei Jahre, 1931 bis 1933.

²⁾ Nur zwei Jahre, 1931 und 1932.

Tabelle 59.
Stackyard Feld.
(Doppelzentner je Hektar)

Jahre	Nach Wicke	Nach Raps	Nach Senf	Mittel der ungedüngten Parzellen 1 und 7
1912 bis 1914 bis 1916 bis 1918 und 1920	24,6	27,6	26,6	15,0
1922 bis 1926	15,1	—	12,6	10,4
1927 bis 1931	14,1	—	11,9	10,5 ¹⁾
1932 bis 1933	23,2	—	19,6	14,0

Dennoch hatte die Grundungung fast keinen Erfolg. die Ertragsdruckung machte sich hier ungefähr im gleichen Maße bemerkbar wie auf ungedüngten Parzellen. (Tab. 59) Das Ergebnis steht im auffallenden Gegensatz zu denjenigen der anderen Versuche, in welchen Reste von Klee und Lupinen die nachfolgende Ernte zweifellos günstig beeinflussten. Zwischen Klee und Senf konnten zwei Vergleichsversuche durchgeführt werden

1. Während des Zeitraumes von 1905 bis 1910 wurde bei den Fruchtwechselversuchen als Grundungung statt Klee Senf verwendet, ein sofortiges und beträchtliches Sinken der Weizen ertrage war die Folge Als Grundungen wieder mit Klee durchgeführt wurden, stiegen auch die Ertrage Der Gesamtertrag in Doppelzentner je Hektar betrug

Jahr	Nach Klee	Jahr	Nach Senf	Jahr	Nach Klee
1886 bis 1889	46,1	1906 bis 1907	57,9	1912 bis 1931	38,2
1890 bis 1893	61,8	1908 bis 1910	28,4		
1894 bis 1897	48,7	1911 bis 1912	20,3		

2. In fünf einzelnen Jahren war Weizen in den Fruchtwechselversuchen nach Klee, im angrenzenden Grundungungsversuch nach Wicke und Senf angebaut worden; die anderen Behandlungsmethoden waren gleich. In allen Fällen waren die Ertrage nach Klee höher als nach einer der anderen Grundungen, sie betrugen in Doppelzentner je Hektar des Gesamtertrages

	Nach Klee	Nach Wicke	Nach Senf
1920	44,5	22,6	23,6
1924	16,8	15,7	17,8
1925	47,7	15,6	10,6
1928	33,9	16,6	17,7
1929	28,4	16,4	14,7
Durchschnitt	34,2	17,3	17,0

¹⁾ 1927 und 1928 Brache

Da Analysen des Ertrages nicht durchgeführt und Proben nicht aufgehoben worden waren, ist es nicht möglich, auch nur annähernd, die Menge des in den Weizenpflanzen enthaltenen und wahrscheinlich von dem Klee stammenden Stickstoffs festzustellen. Wir können sie nur schätzen auf Grund von Warington's durchschnittlicher Stickstoffbestimmung, nach welcher der Stickstoffgehalt der Weizenpflanze 1 kg je Doppelzentner Gesamtertrag beträgt. Danach ergeben sich für den Stickstoff der Pflanzen und für den wahrscheinlich durch Klee ersetzten Stickstoff folgende Werte:

Kilogramm je Hektar jährlich.

1877 bis 1885	1886 bis 1897	1905 bis 1910	1911 bis 1931
90	52	44	38

Unabhängig davon gelangt man zu einer Schätzung aus der Überlegung, daß in 25 Jahren des mittleren Zeitraumes 1886 bis 1910 der auf Klee folgende Weizen beinahe so gute Erträge brachte wie Parzelle 6 der Daueranbau-Reihen mit künstlicher Volldüngung einschließlich 2,8 dz je Hektar Natronsalpeter, eine Menge, die 46 kg Stickstoff entspricht. Natürlich stellt dieser Wert nicht den ganzen durch Klee fixierten Stickstoff, sondern nur einen Bruchteil der von dem Weizen aufgenommenen Menge dar. Wenn die nach Weizen folgenden Früchte in Betracht gezogen werden, scheint der Stickstoff, den der Klee in den Jahren des Wachstums festgelegt hat, ungefähr dreimal so hoch zu sein, etwa 150 kg je Hektar. Aber diese Schätzung ist sehr ungenau.

Die Versuche mit Lupinen lassen sich nicht genau mit den oben beschriebenen vergleichen, weil die Lupinen im Frühjahr angebaut und im Juli untergepflügt werden; unmittelbar danach wurde Kohl ausgesät. Der Versuch wurde so angelegt, um den anteiligen Düngewert der Wurzeln und Stengelteile zu erproben. Nur die Stengelteile haben einen, allerdings sehr ausgeprägten Wert (Tabelle 60).

Tabelle 60.

Kohlernten nach eingepflügten Lupinen, 1934¹⁾.

Eingepflügt	Stickstoff- gehalt der eingepflügten Pflanzen- Substanzen in Kilogramm je Hektar	Kohl- Ertrag in Doppel- zentner je Hektar
1. Nichts (Lupinenwurzeln und Stengelteile entfernt) . .	0	88,7
2. Lupinen, nur Wurzeln	12,6	79,6
3. Lupinen, ganze Pflanze (Wurzeln und Stengelteile) . .	149,6	167,8
4. Lupinen, ganze Pflanze + Stengelteile von Parzelle 2 .	287	212,8

¹⁾ Jahresbericht von Rothamsted 1934, S. 28.

Der Boden in Rothamsted ist viel schwerer als der in Woburn, so daß mit einer ganz verschiedenen Reaktion der Böden auf Gründüngung gerechnet werden mußte. Doch erwies sich sowohl in Rothamsted als auch in Woburn die im Herbst gesäte Wicke gegenüber den nachfolgenden, im Frühjahr gesäten Pflanzen ohne Einfluß; dagegen zeigte im Herbst gesäeter Roggen für Gerste und Zuckerrüben deutlich eine nachteilige Wirkung (Tabelle 61).

Tabelle 61.

Rothamsted: Wirkung der Gründüngung auf verschiedene Kulturpflanzen.
Nach durchschnittlich gleichen Düngungen
ergaben sich 1934 folgende Erträge in Doppelzentnern je Hektar:

	Kartoffeln	Gerste		Zuckerrüben	
		Korn	Stroh	Rüben	Blatt
Keine Gründüngung	148,4	34,2	37,7	317,0	267,0
Wicken, eingepflügt	139,2	34,2	37,8	297,9	228,6
Roggen, eingepflügt	154,2	25,4	31,4	291,1	149,7

Ernte der Gründüngungen am 13. April:

Roggen 126 dz Frischgewicht, 16 dz Trockensubstanz,

Wicken 15 dz Frischgewicht, 1,5 dz Trockensubstanz.

Eine Anzahl in den verschiedenen Teilen des Landes nach dem Versuchsplan der Königlichen Landwirtschafts-Gesellschaft durchgeführte Prüfungen ergaben auch negative Resultate.¹⁾ Das Problem der Gründüngung muß offenbar erst einmal intensiv bearbeitet werden. Diese Düngungsmethode kann nicht eher allgemein empfohlen werden, bis man durch sorgfältige Versuche die für eine erfolgreiche Anwendung der Gründüngung notwendigen Bedingungen kennen gelernt hat.

IV. Die Fruchtwechselversuche: Nachwirkung des Düngers bei Verfütterung von Baumwollkuchen und Mais, 1877 bis 1935.

Diese umfangreichen Versuchsreihen waren angelegt worden, um den Unterschied festzustellen zwischen dem Wert des Stallmistes, der von Tieren mit eiweißreicher Fütterung stammte und solchem, bei welchem die Tiere eiweißärmeres Futter erhalten hatten. Der praktische Wert der Untersuchung lag darin, daß ein Bauer, der seinen Hof aufgibt, wenn er schweres, eiweißhaltiges Futter gegeben hatte, von dem neuen Pächter einen weit höheren Betrag beanspruchen konnte, als wenn er den Tieren nur leichte Futtermittel hatte zukommen lassen.

Die Frage ist nun die: Welchen Vorteil kann der neue Pächter aus diesen Maßnahmen ziehen? Alle sechs Versuchsreihen brachten dieselben Ergebnisse: Ein großer Unterschied konnte nicht festgestellt werden zwischen Nachwirkungen des Stallmistes von Kuchen- und solchem von Maismehlfütterung. Wenn sich dieses Ergebnis als allgemein richtig er-

¹⁾ J. Roy. Agr. Soc., 1926, Bd. 87, S. 296.

weisen sollte, könnte man daraus schließen, daß viele Bauern ungerechterweise Geld bezahlten für etwas, was keinen Wert besaß. Sind die Tabellen, die in Großbritannien zur Festsetzung der Entschädigung für Stallmist-Werte benutzt werden, falsch?

Die Anlage der ersten Versuchsreihen war einfach. Eine Anzahl Schafe und Ochsen erhielt Kuchen aus geschälter Baumwolle, ein anderer Teil der beiden Tierarten bekam Maismehl. Die Futtermengen waren so gehalten, daß sie einen genügenden Unterschied im Stickstoffgehalt der Ausscheidungen gewährleisteten. Der Mist wurde auf zwei verschiedene Arten dem Boden zugeführt. Die Ochsen wurden in Boxen gefüttert, so daß ihre Exkremente als Stallmist für die Rübenparzellen zur Verwendung kamen. Die Schafe dagegen wurden auf Kleeefeldern gepercht und mit Kuchen und Mais gefüttert, so daß ihre Ausscheidungen dem Lande direkt zugeführt wurden. Dieses sind die beiden Methoden, nach welchen in der Praxis die Exkremente der Tiere in Dünger umgewandelt werden, und der Versuch war angelegt worden, um beide Arten zu prüfen. Die allgemeinen Anbaubedingungen entsprechen den sonst üblichen. Zwei Parzellen wurden hinzugenommen, um einen Vergleich zwischen Natronsalpeter und tierischen Ausscheidungen als Düngemittel zu ziehen. Diese Parzellen zeigten die üblichen Unterschiede in den Erträgen, die durch ihren Nitratsvorrat bedingt waren. Die der Parzelle 3 als Natronsalpeter zusätzlich zugeführte Stickstoffgabe von 29 kg je Hektar brachte im Vergleich zu Parzelle 4 72 dz Rüben mehr: während eine zusätzliche Menge von 21,5 kg Stickstoff bei Gerste eine Steigerung von 336 kg Korn- und 896 kg Gesamtertrag je Hektar zur Folge hatte. Diese Mehrerträge waren ganz normal: bei diesen Methoden gab es tatsächlich nichts Ungewöhnliches und es lag auch kein besonderer Grund vor, weshalb ein im Stickstoffgehalt des Stallmistes oder der tierischen Ausscheidungen vorhandener Unterschied sich nicht voll auswirken sollte.

Durch Versuchsreihen in den Jahren 1886 bis 1897 konnte man an drei Fruchtarten den Einfluß des Pferchens beobachten, d. h. Verfüttern der Rüben auf dem Lande, wo sie gewachsen waren, so daß die Exkremente der Tiere den Boden düngen konnten. Die Versuchsergebnisse betrugen:

In Doppelzentner je Hektar jährlich.

		Ohne Pferchen ungedüngt	Mit Pferchen ungedüngt
		Parzellen 5 bis 8	Parzellen 1 und 2
Gerste, Gesamtertrag	34,8	48,4
Kornertrag	15,9	21,9
Kleeheu	47,4	55,0
Weizen, Gesamtertrag	52,2	56,0
Kornertrag	21,2	22,4

Das Pferchen hatte sowohl auf die Gerste als auch auf den in ihr eingesäten Klee einen günstigen Einfluß; der Erfolg bei Weizen könnte auch als ein Ergebnis des verbesserten Kleewachstums angesehen werden.

In den Versuchsserien der Jahre 1886 bis 1897 schien der Mist der Tiere mit Baumwollkuchenfütterung als Düngemittel eine stärkere Nachwirkung zu erhalten als derjenige von Tieren mit Maismehlfütterung; aber dieser Unterschied zeigte sich nur bei Gerste und hatte auf keinen Fall irgendeine statistische Bedeutung. Die Parzellen, auf welchen Tiere gepfercht waren, brachten folgende in Doppelzentner je Hektar berechneten Ergebnisse:

	Mist nach Verfütterung von Baum- wollkuchen Parzelle 1	Mist nach Verfütterung von Maismehl Parzelle 2	Mist von Tieren, die weder Kuchen noch Mehl erhalten hatten. Künstliche Düngegaben Parzelle 3 und 4	
Gerste, Gesamtertrag	50,8	46,0	55,8	47,3
Kornertrag	22,9	21,0	25,0	21,0
Kleeheu	53,9	56,1	60,7	57,8
Weizen, Gesamtertrag	56,9	55,0	55,6	54,9
Kornertrag	22,7	22,0	22,4	22,0

Den Einfluß der Stallmistdüngung auf Rübenparzellen — allerdings nicht auf andere — kann man aus den Versuchsreihen der Jahre 1904 bis 1911 abschätzen; für die drei Jahre mit Rübenbau (1908 bis 1910) liegen folgende Ergebnisse vor:

Rübenerträge in Doppelzentner je Hektar.

Ungedüngt (Mittlere Erträge der Parzellen 1 bis 4)	Stallmistdüngung ¹⁾ (Mittlere Erträge der Parzellen 5 bis 8)
256,5	344,6

Einzelserträge der Stallmistparzellen.

Baumwoll- kuchen- Fütterung Parzelle 5	Maismehl- Fütterung Parzelle 6	Weder Kuchen- noch Mehl- Fütterung Parzelle 7	Weder Kuchen- noch Mehl- Fütterung Parzelle 8
335,6	333,8	353,4	356,2

Man konnte also eine durch Stallmistdüngung hervorgerufene Ertragssteigerung von 87 Doppelzentner je Hektar, aber keinen Unterschied zwischen den verschiedenen Stallmistarten feststellen. Das Vorhandensein irgendwelcher Nachwirkung ließ sich nicht beweisen.

In einem Punkte wich dieser Versuch von den in der Praxis üblichen Bedingungen erheblich ab. Die innerhalb von vier Jahren einmal angewendete Stallmistgabe war stets sehr gering, nur 75 bis 100 Doppelzentner je Hektar. Die beim Feldbau gewonnenen Erfahrungen lehren übereinstimmend, daß derartige geringe Düngermengen nicht benutzt werden sollen. Und tatsächlich zeigte sich in den Versuchsreihen mit dauerndem

¹⁾ Etwa 100 dz je Hektar.

Ein in Rothamsted neun Jahre (1904 bis 1912) lang durchgeführter Vergleich zwischen Dünger von Kuchenfütterung und gewöhnlichem Dünger.

Gesamtertrag der ungedüngten Parzelle je Hektar = 100.

	1. Ernte (Jahr der An- wendung)	2. Ernte	3. Ernte	4. Ernte
Dünger von Kuchenfütterung . .	173	138	120	113
Dünger, keine Kuchenfütterung gegeben	144	135	126	117

Weizen- und Gerstenbau eine Stallmistdüngung von 75 oder 100 Doppelzentner je Hektar erst dann wirksam, als sie vier Jahre nacheinander zur Anwendung kam. In einem späteren Versuch in Rothamsted, bei welchem größere Düngergaben (400 dz je Hektar) benutzt wurden, wurde Mist von Kuchenfütterung mit solchem ohne Kuchen- oder Maisfutter verglichen. Hier erwies sich der Mist von Kuchenfütterung als bedeutend besser, allerdings nur im ersten Jahr; im zweiten und folgenden Jahr zeigten sich die beiden Arten ähnlich, obgleich beide wirksam waren.

Das Ergebnis von Woburn ähnelt dem von Rothamsted im zweiten Jahr nach der Anwendung.

Der Versuch zeigt, daß Stallmistdüngung günstige Wirkungen besitzt, die nicht der Bereicherung des Mistes durch Kuchenfütterung zugeschrieben werden können. Die Wirksamkeit des Stallmistes bedarf noch weiterer Untersuchungen. Dem Mist fehlt die für die günstige Beeinflussung des Pflanzenwachstums notwendige zusätzliche Stickstoffmenge; Natronsalpeter dagegen behält seine normale Wirksamkeit bei. Diese Tatsache läßt sich nur mit einem Stickstoffverlust oder einem Unwirksamwerden des Stickstoffs erklären. Mit einem Verlust durch Auslaugen der Felder, wo Schafe gepfercht worden sind, kann man rechnen, weil die Früchte dieser Felder in den Wintermonaten verbraucht werden, in denen das Durchsickern sehr stark ist. Es kann sowohl eine Verdunstung des Ammoniaks aus dem Boden als auch ein mikrobiologisches Freiwerden von gasförmigem Stickstoff stattfinden, obgleich bei Feldversuchen Vorgänge dieser Art nicht sicher bestätigt worden sind. Mikrobiologische Bindung von Ammoniak und Nitrat an organische Substanzen und Umwandlung in komplexe und unverwertbare stickstoffhaltige Substanzen sind bekannte Tatsachen.

Nach der Art der Versuchsanstellung konnte Natronsalpeter bei weitem nicht im gleichen Umfange wie die tierischen Ausscheidungen von diesen Verlusten betroffen werden und konnte infolgedessen besser seine normalen Wirkungen erhalten.

Dieses starke Nachlassen der Wirksamkeit des Stickstoffs in Gegenwart von organischen Substanzen, das sowohl bei den Fruchtwechsel- als auch bei den Gründungsversuchen auftrat, soll durch neue Feldversuche weiter erforscht werden.

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THE WEED SEED POPULATION OF ARABLE SOIL

III. THE RE-ESTABLISHMENT OF WEED SPECIES AFTER REDUCTION BY FALLOWING

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(With eight Figures in the Text)

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I. INTRODUCTION

FROM the practical point of view the most important feature of weed reduction by various methods of treatment is the length of time that such reduction remains effective. Special methods of eradication involve extra expense, and, if fallowing is carried out, loss of crops as well. If pernicious weeds can be drastically reduced by such methods, the expense and trouble involved is justified from an economic point of view provided the number of such weeds remains at a low level for several succeeding years. If, however, the weeds re-establish themselves very rapidly it is probable that the increased crop resulting from the temporary reduction of competition will not compensate for the extra outlay. Hitherto practically no quantitative information has been available as to the behaviour of weeds after such drastic methods of reduction as fallowing. Most cultivators have their own ideas on the subject, and divergent opinions are held owing to differences in local conditions and the varying quality of the observations made.

For the past eleven years, since autumn 1925, a continuous quantitative experiment has been carried out on Broadbalk wheat field at Rothamsted, in which the potential weed flora has been estimated by recording the weed seedlings obtained from numerous soil samples taken year after year from the same areas immediately after harvest. During the first four years all parts of the field were subjected to either two or four years' consecutive fallow, the whole area returning into crop in the autumn of 1929. In addition to the fallowing, special attention was devoted to the eradication of weeds among the crop, in the hope of effecting a real reduction by a combination of the two methods of control. Two previous papers have demonstrated the enormous potential weed flora, as indicated by the number of living seeds present in the soil before active measures were taken, and the comparative effect of fallowing and cultivation among the crop on the numbers of these viable seeds (Brenchley and Warington, 1930, 1933). In nearly every case adequate fallowing caused a more or less considerable decrease in the potential flora, but the effects of crop cultivation varied widely, from a decrease equal to that caused by fallowing to an increase to two or three times the original number.

The methods used in obtaining the seedlings and in handling the large numbers of figures entailed were fully described in the first paper (Brenchley and Warington, 1930, pp. 238-41), to which reference should be made. Data are now available showing the after-effects of fallowing for varying periods, when the land has again been cropped for several successive seasons, but before these can be dealt with profitably, certain other points need discussion.

II. EFFECT OF TIME OF FALLOWING ON THE WEED FLORA

Early ploughing of the stubble encourages germination, and many of these seedlings are probably killed by winter conditions, while the great majority of the remainder are cut down by spring cultivations before they flower and ripen seeds. This is true for all except a very few species, notably *Capsella bursa-pastoris*, *Arenaria serpyllifolia*, *Veronica buxbaumii* and *Poa annua*, which are able to reach the seeding stage very rapidly even in the autumn and winter months, and so can maintain their numbers of seeds in the soil in spite of repeated later cultivations.

Under normal conditions fallowing operations begin by cultivating or ploughing the stubble as soon as possible after harvest. When the time arrived to begin the fallowing of the second half of Broadbalk field, the area was left untouched, owing to unavoidable circumstances, from harvest in August 1927 till March 1928. The first ploughing was then done, and fallowing operations carried on in the normal way. The delay in ploughing influenced the behaviour of various species in different ways, so that in some cases the results of the first year under fallow do not correspond with those obtained previously during the parallel period on the other part of the field.

When the autumn stubble ploughing was omitted in 1927, an opportunity was provided for several weed species to increase their stocks of seed in the soil (Table I). Weeds were plentiful in the stubble, and many of them doubtless

Table I. *Number of viable buried seeds in successive years (all seven plots together)*

	Actual population per 14 sq. ft.				Relative population in percentages			
	1927	1928	1929	1930	1927	1928	1929	1930
Grass, chiefly <i>Poa annua</i>	22	171	49	139	100	777	223	632
<i>Bartsia odontites</i>	20	62	37	131	100	310	185	655
<i>Capsella bursa-pastoris</i>	307	893	380	561	100	290	124	183
<i>Senecio vulgaris</i>	96	148	34	19	100	154	35	20
<i>Arenaria serpyllifolia</i>	269	406	211	333	100	151	78	124
<i>Atriplex patula</i>	124	186	15	34	100	150	12	27
<i>Stellaria media</i>	29	42	31	78	100	145	107	269
<i>Matricaria inodora</i>	20	28	16	28	100	140	80	140
<i>Polygonum aviculare</i>	44	55	15	32	100	125	34	73
<i>Veronica hederaefolia</i>	296	345	134	246	100	117	45	83
<i>V. buxbaumii</i>	361	404	130	77	100	112	36	21
<i>Euphorbia exigua</i>	21	23	6	18	100	110	29	86
<i>Anagallis arvensis</i>	24	26	17	11	100	108	71	46
<i>Legousia hybrida</i>	168	168	98	284	100	100	58	169
<i>Linaria minor</i>	55	51	46	23	100	93	84	42
<i>Scandix pecten</i>	16	14	1	9	100	88	6	56
<i>Caucalis arvensis</i>	80	65	46	49	100	81	58	61
<i>Polygonum convolvulus</i>	9	7	1	2	100	78	11	22
<i>Papaver</i> spp.	39,078	29,037	17,407	23,031	100	74	45	59
<i>Veronica arvensis</i>	1,204	867	522	1,484	100	72	43	123
<i>Alchemilla arvensis</i>	2,720	1,868	1,213	4,431	100	69	45	163
<i>Aethusa cynapium</i>	36	22	15	14	100	61	42	39
<i>Medicago lupulina</i>	99	57	34	153	100	58	34	155
<i>Sonchus arvensis</i>	54	25	7	7	100	46	13	13
<i>Galium aparine</i>	228	69	28	69	100	30	12	30
<i>G. tricornis</i>	16	4	2	7	100	25	13	44
<i>Alopecurus agrestis</i>	6,562	1,253	342	3,723	100	19	5	57
<i>Myosotis arvensis</i>	349	60	34	145	100	17	10	42

continued to flower and seed until they matured or were cut down by frost. This category includes *Anagallis*, *Atriplex*, *Bartsia*, *Euphorbia*, *Matricaria*, *Polygonum aviculare* and *Veronica buxbaumii*. Another set of species apparently increased their seed stocks by the germination and rapid development of some of their shed seeds which were lying sufficiently near the surface. *Arenaria*, *Capsella*, *Poa annua*, *Senecio* and *Stellaria* will all germinate and flower freely in the early autumn, and *Veronica hederaefolia* flowers and fruits abundantly in the very early part of the year. In all these cases the increase of seed in the soil was so large that fallowing operations from March to August failed to reduce them to their level at the previous harvest time. *Legousia* is more difficult to explain, as it has usually finished fruiting by July, and growing plants are seldom seen among the stubble. Normally, however, it germinates early, and possibly if the optimum period for germination had passed by the time of the first ploughing, practically all the seeds might have remained dormant in the soil until their favourable growing season again came round.

Proof of such occasional behaviour has already been reported in the case of *Alchemilla* (Brenchley and Warington, 1930, p. 247).

The remainder of the weed species were reduced by fallowing to a greater or less degree in spite of the delay in ploughing. This does not necessarily imply that none of these species continued to ripen seed, but rather that any increase thus effected was less than the reduction by after-cultivation. *Alopecurus*, *Papaver*, and *Sonchus*, however, are species which would be unable to increase their seed stocks to any extent during the resting time before ploughing owing to their habit of growth. All have a long vegetative period before flowering, and when a crop of seed has ripened, another year elapses before the next generation has reached the same stage, so that the delay in ploughing would not affect the efficiency of the first year's fallow with these species. The varying response of the weed species to delayed ploughing is thus not connected with the general abundance or scarcity of any particular species, but is obviously associated with habit. It happens that *Alopecurus* and *Papaver*, which together provided 87 per cent. of the total seed flora, are both species which have a long growing period before they flower and ripen seed in the summer, while *Alchemilla* and *Veronica arvensis*, which contributed a further 8 per cent., are also late-flowering species which are practically over by harvest time. In the remaining twenty-four species, contributing only 5 per cent. to the total, the increase or decrease in seed population during the season 1927-8 fluctuated as much in the scarcer species as in the more abundant (Table II).

Table II. *Relative prevalence of weed species*

Area 14 sq. ft.		
	Number of seeds in 1927	Percentage of total seeds
<i>Papaver</i> + <i>Alopecurus</i>	45,640	87
<i>Alchemilla</i> + <i>Veronica arvensis</i>	3,924	8
24 other species	2,743	5

The importance of promptitude in beginning operations after harvest is further illustrated by grouping the various weed species according to their relative seed populations after one year under fallow, and comparing the results of immediate and delayed ploughing.

Table III. *Summary of effect of fallowing for one year on percentage of weed seeds relative to number originally present in soil*

	Fallowed 1925-6 (ploughed autumn)	Fallowed 1927-8 (ploughed spring)
Over 100%	4 species (105-136%)	13 species (108-777%)
81-100%	—	4
41-80%	8	7
40% and under	16	4

Not only was the general degree of reduction much less in the 1927-8 fallow, but nearly half the species were definitely increased, whereas this only occurred with a very few species in the earlier fallow.

III. BEHAVIOUR OF WEED FLORA WHEN LAND WAS CROPPED AFTER FALLOWING

The first crop of wheat after the two- or four-year fallow was heavy and considerably above the normal on all plots, the increase being attributed to the extra fertility accumulated during the fallow period. This initial improvement was not maintained, but the second and succeeding crops reverted to a lower level determined by the seasonal conditions. This is shown in Table IV, which gives the successive crops up to the time that another system of periodical fallowing was begun on the different sections of the field.

Table IV. *Average wheat crop on the seven plots used in the weed-seed experiment*

	Bushels of grain per acre	
	Top part of field (sections 1 and 2)	Bottom part of field (sections 4 and 5)
1925	15.4	15.6
1926	F.	5.3
1927	F.	15.5
1928	49.5	F.
1929	21.3	F.
1930	8.4	30.8
1931	F.	19.3
1932	F.	14.2

F. — fallow. Figures in heavy type show first crop after fallow.

(a) *Influence of first year's crop after fallow on weed flora*

It would naturally be expected that the heavy wheat crop would so dominate the situation that its competition would prevent the weed flora from reasserting itself to any considerable degree during the first year. As the wheat crop is determined in bushels of grain, which might be translated into the number of seeds produced per acre, so, too, the weed crop may fairly be estimated by the number of living seeds found in the soil at the end of the growing season. With many species the number of these seeds showed either little change or a relatively small increase or decrease by the end of the first year's cropping. The species may be arranged in three groups, according to their general response to the new conditions. In the first group *Aethusa*, *Anagallis*, *Atriplex*, *Caucalis*, *Galium tricornue*, *Sonchus* (Fig. 1) and *Polygonum aviculare* (Fig. 2) behaved very similarly after both periods of fallowing, all being species which had been reduced to very small numbers, and which remained at a low level. *Euphorbia*, *Linaria*, *Matricaria* and *Papaver* (Fig. 2) showed more variation in their behaviour, any increase or decrease not being coincident in the two series. Here again the numbers after fallow were low, with the exception of *Papaver*, which was by far the most abundant throughout. It is easy to understand the failure of species to overcome the competition of the heavy wheat crop when only a few seeds were available for the purpose,

but it is more surprising that *Papaver*, with its large stock of seed that survived the fallowing, should not have been able to re-establish itself to a greater

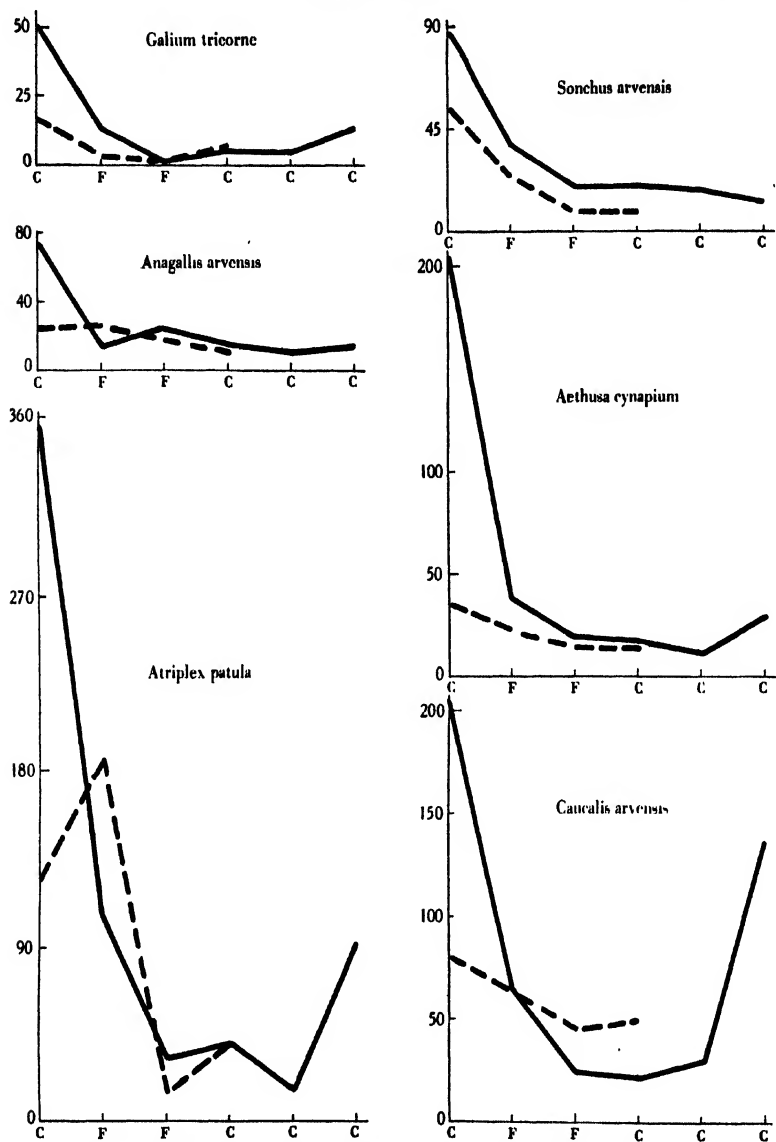


FIG. 1. Number of seedlings in 14 sq. ft. (seven plots together). fallowed —, 1925-7; ---- 1927-9; C, land cropped; F, land fallowed.

extent. This, however, is probably correlated with the fact that *Papaver* is not at all prevalent on the heavy land in this district and so may be constitutionally less able to re-establish itself as rapidly as other species.

A second group of weeds proved their ability to withstand the competition of the wheat and had replenished their stores in the soil to a very considerable

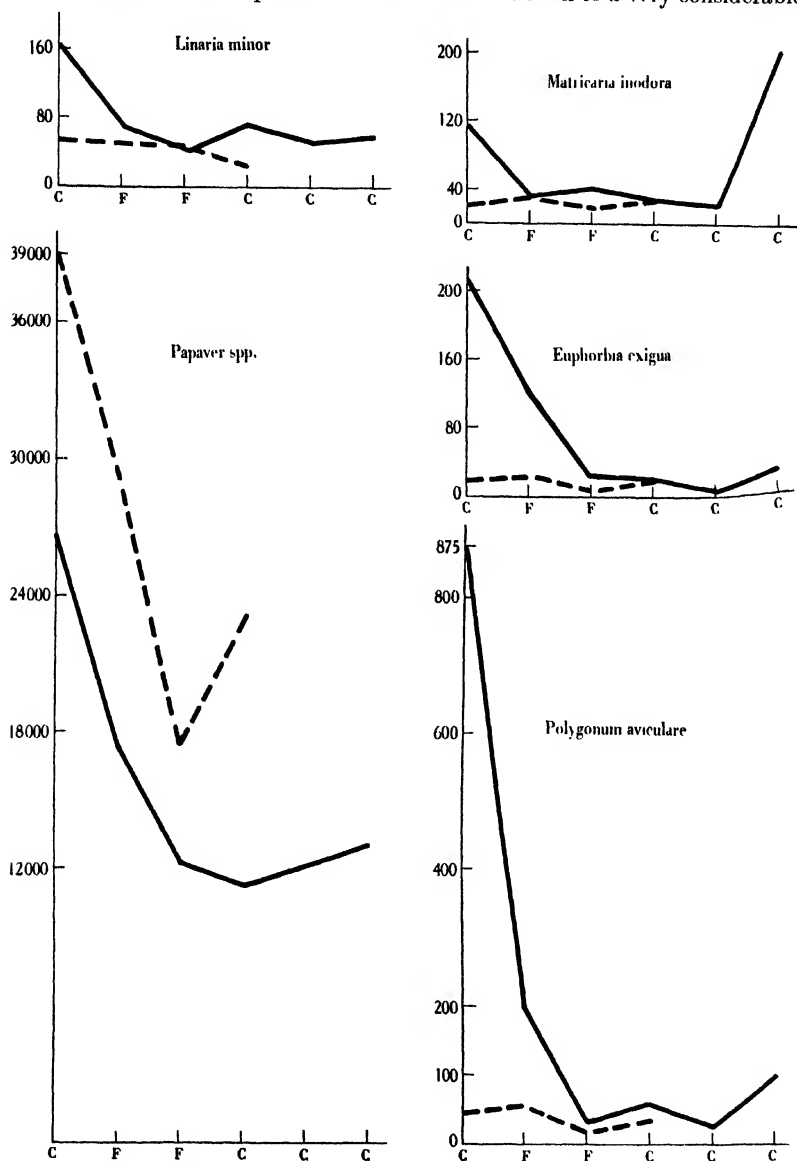


FIG. 2. Number of seedlings in 14 sq. ft. (seven plots together) fallowed — —, 1925-7; 1927-9; C, land cropped; F, land fallowed.

extent by the time the first crop was harvested. *Alopecurus*, *Capsella*, *Myosotis* and *Veronica hederaefolia* (Fig. 3) showed definite increases, though the

numbers did not always reach those present before fallowing began. *Alchemilla*, *Bartsia*, Grass spp., *Stellaria* and *Veronica arvensis* (Fig. 4) showed heavier

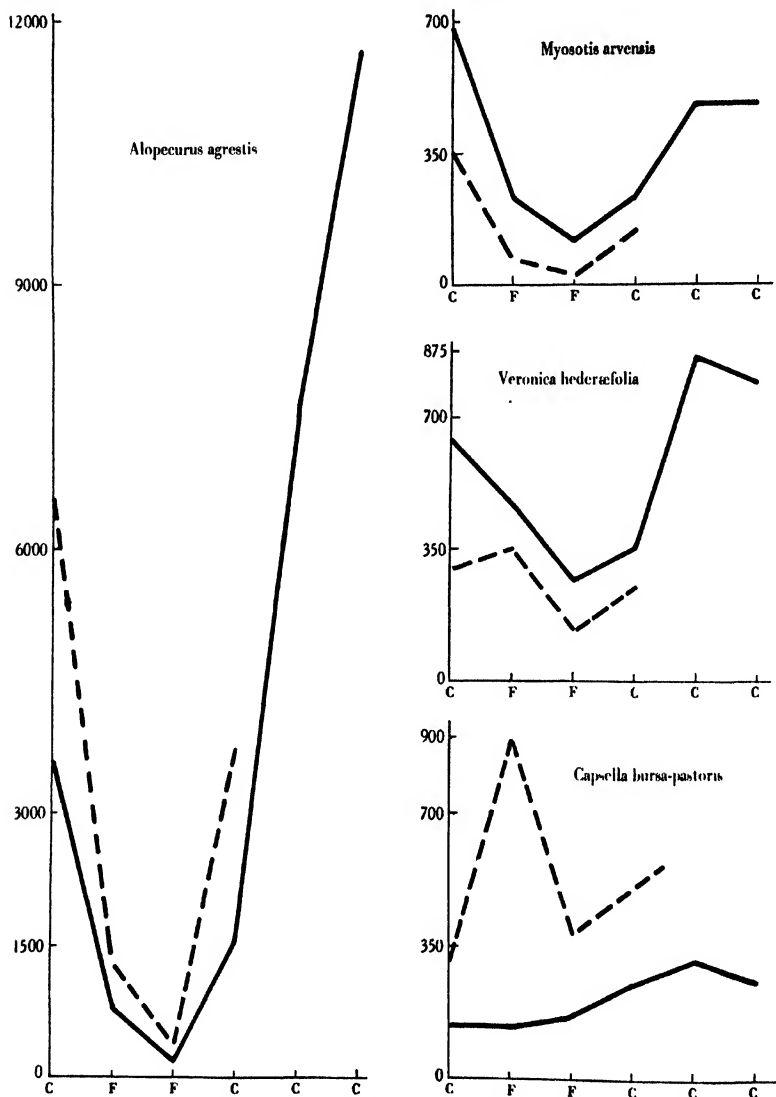


FIG. 3. Number of seedlings in 14 sq. ft. (seven plots together). fallowed —, 1925-7; 1927-9; C, land cropped; F, land fallowed.

proportionate increases, considerably exceeding the original number before the fallow in the first three species. In all these the response was similar after

both periods of fallow. With most of them, the habit of the weeds may account for their success. *Capsella* and Grass spp. (chiefly *Poa annua*) can flower and

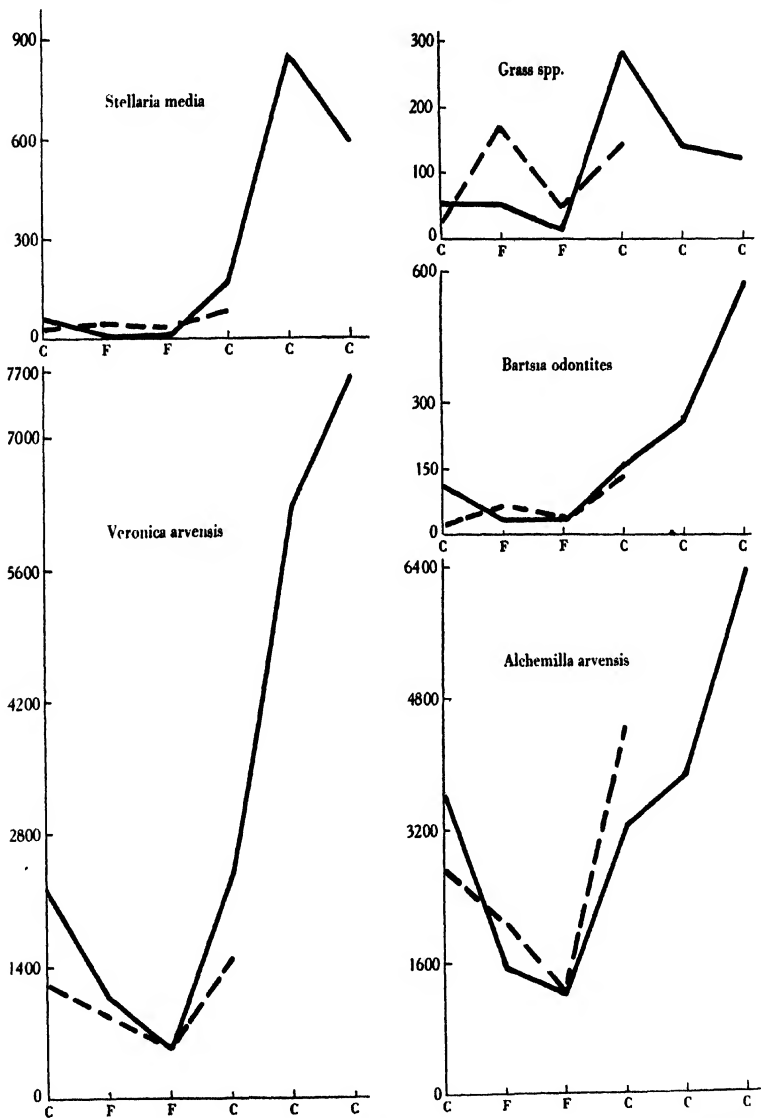


FIG. 4. Number of seedlings in 14 sq. ft. (seven plots together). fallowed ----, 1925-7; 1927-9; C, land cropped; F, land fallowed.

seed all the year round, and so would be able to restock the soil with seed during the autumn and winter months when the growth of the wheat was as yet

insufficient to bring it into effective competition. *Alchemilla*, *Myosotis*, *Stellaria*, *Veronica arvensis* and *V. hederifolia* are all species in which the

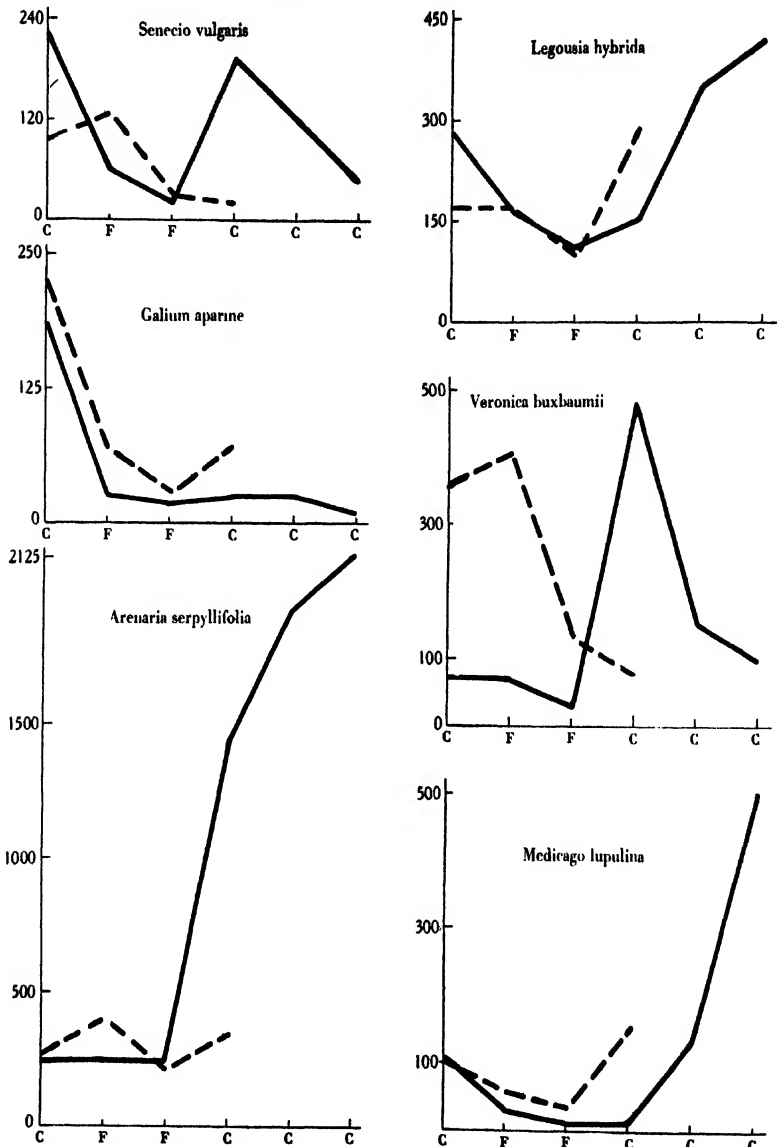


FIG. 5. Number of seedlings in 14 sq. ft. (seven plots together). followed —, 1925-7; - - - - 1927-9; C, land cropped; F, land fallowed.

earlier flowers ripen seed long before the plant reaches maturity, and here again seed formation had probably begun before the wheat competition came

much into action. *Alopecurus* is the only late-flowering upright species in this group, and its success is probably due to the fact that its habit of growth is closely parallel to that of the wheat. Crop and weed germinate and grow up together, flowering at much the same time, and sufficient *Alopecurus* plants must have developed from the relatively small stock of seed left after fallowing to lay the foundation for a rapid recolonisation.

In a third group are the species whose response to cropping varied more widely after the two fallow periods. *Arenaria*, *Senecio* and *Veronica buxbaumii* (Fig. 5) showed heavy increases after the 1925-7 fallow, but *Arenaria* rose only very slightly after the 1927-9 period, while the other two species diminished in number. On the contrary, *Galium aparine*, *Legousia* and *Medicago* (Fig. 5) improved their position more after the second fallow than after the first. It is not possible to suggest a reason for the divergent behaviour of these species, though it was probably connected with the seasonal conditions that occurred at critical times in development.

(b) *Prevalence of weed species in second and subsequent years after fallowing*

To a great extent the behaviour of the various weed species during the first year after fallow was continued for the next two years. Most species which failed to reassert themselves during the first year remained at a low level afterwards, whereas the majority of those which had shown a distinct upward tendency continued to increase more or less considerably, this behaviour being independent of the actual proportion of any species in the total population. The weeds may be classified in groups according to their response during the three years after fallow (cf. Table I and Figs. 1-5).

(1) Little or no recovery throughout: *Aethusa*, *Anagallis*, *Euphorbia*, *Galium aparine*, *G. tricornis*, *Linaria*, *Papaver*, *Sonchus*.

(2) Little or no recovery in first two years, then an increase: *Atriplex*, *Caucalis*, *Matricaria*, *Polygonum aviculare*, *Scandix*.

(3) Increase in first two years, then fall or steady in third: *Capsella*, *Myosotis*, *Stellaria*, *Veronica hederifolia*.

(4) Steady increase throughout: *Alchemilla*, *Alopecurus*, *Arenaria*, *Bartsia*, *Legousia*, *Medicago*, *Veronica arvensis*.

(5) Quite irregular in behaviour: Grass spp., *Senecio*, *Veronica buxbaumii*.

This grouping applies to the response after the 1925-7 fallow for which exact data are available, and the indications from field observations after the later fallows are that on the whole the general trend of behaviour of the various species is similar.

Papaver and *Galium aparine* showed a greater early increase, and *Arenaria* a less marked recovery in the first year after the 1927-9 fallow, which may have influenced their later response to some extent.

The varying rate of recolonisation has resulted in a definite change in the balance of the weed flora, as some previously plentiful species have now become relatively insignificant, while others have come into much greater prominence. The persisting reduction of *Papaver* is the dominant feature in this change of balance, as the numbers are so large as to mask the more rapid increase of the rest. The steady reduction in the total seeds present in the soil caused by fallowing was followed by an equally steady increase year by year.

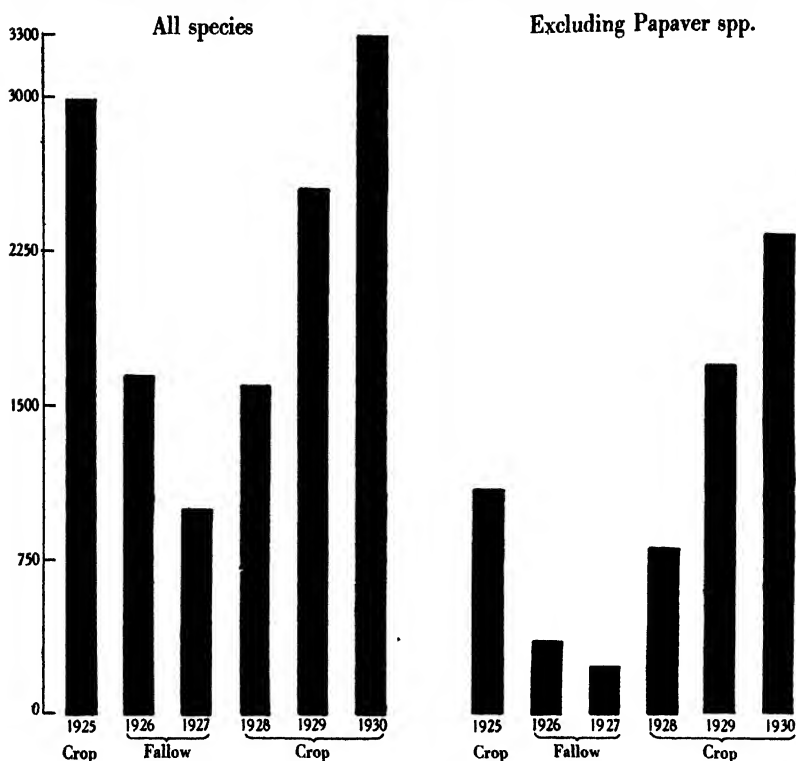


FIG. 6. Average number of weed seeds per sq. ft. (seven plots together), showing re-establishment of weeds during the first three years after fallowing.

After the third wheat crop the total number of buried weed seeds had reached and slightly exceeded the number present at the time fallowing operations began. From this point of view the beneficial effects of fallowing persisted for two years. Leaving *Papaver* out of consideration, however, the recolonisation by all other species was much more rapid. After the first crop the number of weed seeds had closely approached, and after the second crop far exceeded, the original stock present before fallow, the rise being continued in the succeeding year (Fig. 6). This rapid increase in weed seeds coincided with a parallel decrease in the yield of wheat, but no opinion can be expressed as to

how far the relative movements of crop and weeds were correlated, especially as reduction in crop did not induce a corresponding rise in *Papaver*. As regards weed reduction, however, fallowing is for most species only a temporary expedient. There is also a certain risk that some species may profit by the after-fallow conditions to such an extent that their increase may be so rapid as to make them a serious menace after the lapse of a very few years. The critical period seems to be the behaviour during the first year of return into crop, when the future line of development of most species is apparently determined.

Fallowing operations were primarily intended to reduce *Papaver* and *Alopecurus*, and were successful for *Papaver*, but the return of *Alopecurus* was unduly rapid. This is well shown in Fig. 7 which illustrates from left to right



FIG. 7. Second crop of weeds from samples taken after harvest in 1929. Left to right. 16 3 A, after four years under fallow; 16 4 A, after two years under fallow; 16 2 C, after two years in crop, following two years under fallow.

the scarcity of seedlings after four and two years fallow, and the abundance of *Alopecurus*, with other species, after two years in crop following two years fallow. The sample in each case represents only one quarter of a square foot of soil in area. The competition factor of *Alopecurus* has always been considered to be high, and a detailed comparison of crop yield and the number of *Alopecurus* seeds was made to see how far the two species reacted on one another. Complete data were available between 1925 and 1933 for seventy-seven cases in which wheat followed wheat on the various sections of the seven plots under examination. In fifty-five of these cases, as the wheat yield increased, the number of *Alopecurus* seeds decreased, and *vice versa*, but in the remaining twenty-two instances the crop yield and *Alopecurus* seeds moved in the same direction, both increasing or decreasing simultaneously. It would, therefore, seem that while the two species usually come into active competition, conditions frequently occur which supersede the competition factor and either

encourage or discourage wheat and *Alopecurus* alike. A few random instances may be quoted (Table V) to show that the divergence of the figures is quite outside the bounds of experimental error and also that the necessary conditions cannot be seasonal, as the yields of *Alopecurus* and wheat may run both parallel and in opposition on different parts of the same plot in the same year.

Table V. *Increase or decrease of yields of wheat and Alopecurus*

Plot	Section	Year	Running parallel		Plot	Section	Year	Running in opposition	
			Crop Bushels of grain per acre	<i>Alopecurus</i> No. of seeds per sq. ft.				Crop Bushels of grain per acre	<i>Alopecurus</i> No. of seeds per sq. ft.
5	5	1926	2.2	190	5	5	1925	7.7	123
		1927	6.5	255			1926	2.2	190
7	4	1930	34.6	503	7	3	1930	38.9	39
		1931	20.2	375			1931	27.0	117
12	1	1929	22.9	955	12	2	1929	22.9	596
		1930	6.4	852			1930	9.7	1836
16	2	1929	26.3	1251	16	1	1929	26.3	635
		1930	6.0	924			1930	9.9	1767
18	4	1925	14.2	614	18	5	1926	3.6	400
		1926	3.6	273			1927	15.3	243

(c) *Effect of prolonged fallowing upon the subsequent weed flora*

So far no account has been given of the section of Broadbalk field which was left uncropped from 1925 to 1929, the cultivation during this period being identical with that of the area under a two-year fallow in the same year. The response of the crop was similar after both long and short fallows, heavy yields being obtained in the first year, followed by a considerable drop in the second. The average yields of grain on adjacent sections of the seven experimental plots were as follows:

	After 4 years fallow (bushels per acre)	After 2 years fallow (bushels per acre)
1930	39.0	36.3
1931	22.6	21.8
1932	17.7	15.2
1933	26.2	25.6

The competition offered by the crop to the weeds was, therefore, very much the same in both sections, and it will be seen later that the weed response was also very parallel.

The check in weed seed reduction due to delayed cultivation in 1927-8 described on p. 481 was also shown in the course of the long fallow. Species that were able to increase their number before the delayed fallowing operations were begun, were equally able to do so where the land had already been under fallow for two years and no fresh seeds were available. A comparison of Table I with Table VI shows clearly the parallel behaviour of the majority of the species concerned. The species for 1928 in Table VI which show a definite

increase after two years fallow, correspond almost exactly with those for the same year in Table I where fallowing was only beginning.

Table VI. *Number of viable weed seeds on area fallowed for four successive years (all seven plots together)*

	Actual population per 7 sq. ft.					
	Crop		Fallow		Crop	
	1925	1926	1927	1928	1929	1930
Grass sp. (chiefly <i>Poa annua</i>)	26	32	9	90	50	233
<i>Bartsia odontites</i>	18	15	10	5	10	26
<i>Capsella bursa-pastoris</i>	80	109	60	229	64	119
<i>Senecio vulgaris</i>	150	45	8	106	53	40
<i>Arenaria serpyllifolia</i>	138	156	111	154	100	186
<i>Atriplex patula</i>	95	24	21	14	3	13
<i>Stellaria media</i>	42	6	1	57	64	252
<i>Matricaria inodora</i>	11	34	4	9	2	6
<i>Polygonum aviculare</i>	258	89	15	20	—	21
<i>Veronica hederæfolia</i>	268	239	111	109	37	57
<i>V. buxbaumii</i>	44	54	23	113	45	42
<i>Euphorbia exigua</i>	101	53	11	6	—	4
<i>Anagallis arvensis</i>	21	7	3	5	2	1
<i>Legousia hybrida</i>	278	158	140	84	58	199
<i>Linaria minor</i>	36	34	9	9	1	6
<i>Scandix pecten</i>	223	34	3	4	—	—
<i>Caucalis arvensis</i>	68	37	17	22	6	4
<i>Polygonum convolvulus</i>	18	5	1	—	—	—
<i>Papaver</i> spp.	18,001	10,959	8,280	6,063	3,760	5,319
<i>Veronica arvensis</i>	1,045	472	198	137	103	279
<i>Alchemilla arvensis</i>	1,980	942	622	532	269	1,002
<i>Aethusa cynapium</i>	77	31	4	5	2	5
<i>Medicago lupulina</i>	47	15	2	4	8	1
<i>Sonchus arvensis</i>	38	8	4	8	3	3
<i>Galium aparine</i>	56	8	5	—	—	—
<i>G. tricornis</i>	16	7	5	—	1	—
<i>Alopecurus agrostis</i>	1,809	437	87	93	29	624
<i>Myosotis arvensis</i>	90	51	19	12	7	28

Figures in heavy type indicate increase in seed population after delayed cultivation of fallow. The species are arranged in the same order as in Table I to facilitate comparison.

With few exceptions the numbers increased, decreased or remained stationary after the first year's return into crop, regardless of the length of the previous fallowing. *Scandix*, *Galium aparine*, *Galium tricornis* and *Polygonum convolvulus* appeared to be eliminated by the long fallow as not a single seedling appeared. *P. aviculare*, on the other hand, increased apparently from nothing after the long fallow, indicating a rapid increase from a residual stock of seed which was so small that no representative appeared in the samples taken in the last uncropped year. *Medicago* and *Linaria* were the only species that varied strikingly in behaviour with the different periods of fallowing. Both remained in fair quantity at the end of the two years fallowing period, and under the first crop *Medicago* was multiplied several times whereas *Linaria* was further decreased. The longer fallow reduced them both far more heavily, but the ultimate behaviour was reversed, as *Linaria* improved its position while *Medicago* became still less plentiful. This depression with *Medicago* continued with the second wheat crop in 1931, and it was not till 1932 that

it began to make any progress, though it still remained much behind that on the area that had been under short fallow. Figures for this later period are available for three plots, on only one of which (plot 18) *Medicago* occurs in any appreciable amount.

Table VII. *Behaviour of Medicago lupulina after long and short fallows*

	Plot 18. Average number of seeds per sq. ft.			
	Last year of fallow 1929	Crop		
		1930	1931	1932
Long fallow, 4 years	1	—	1	47
Short fallow, 2 years	4	16	45	108

Generally speaking, therefore, the ultimate behaviour of most weed species is not affected by the length of time the land is repeatedly cultivated under fallow, as in most cases the increase or decrease under crop is identical in direction. When the cultivations are carried out at the right time to check any species from producing fresh seed, the population is reduced more heavily with a longer period of fallow, and it takes longer for any particular species to regain its original position. It must be realised, however, that under normal farming conditions such prolonged fallowing is impracticable, the usual period being one year or less, but in the present instance the weedy condition of the experimental wheat field demanded special treatment, thus providing a unique opportunity for investigating the question of the behaviour of seeds present in the soil.

IV. OBSERVATIONS ON PERIODICITY OF GERMINATION AND LENGTH OF NATURAL DORMANCY OF BURIED WEED SEEDS

In the first paper of this series a distinction was drawn between the "natural" and "induced" dormancy of seeds. A seed may be said to be naturally dormant if it will not start into growth when it is placed in conditions favourable to germination, whereas "induced" dormancy is that state forced on a seed when it is capable of immediate germination but finds itself in circumstances that are unfavourable to growth, as when seeds are buried too deeply, or soil conditions are too arid. The aim of fallowing operations is to prevent induced dormancy and to bring as many buried seeds as possible to the surface where they can germinate if they are ready. In the laboratory experiment the fallowing conditions are intensified, as the soil is disturbed regularly and frequently and favourable growth conditions are maintained throughout. In spite of this many seeds have remained inert in the seed pans for several years. More than fifty of the total samples taken in 1925, 1926 and 1927 were retained after the normal three-years period, and these have since been treated exactly the same as more recent samples and are still under observation. Altogether an additional 1610 seedlings have appeared,

representing twenty-one species, of which five have only contributed one or two individuals. The proportion of these later seedlings to the total number of germinated seeds is shown in Table VIII.

Table VIII. *Prolonged natural dormancy*

Species	Total No. of seeds recorded from samples	Viable seeds remaining dormant for more than 3 years		Longest period of dormancy recorded till Dec. 31st, 1935 Years
		No.	%	
<i>Achutha cynapium</i>	126	41	32.5	10
<i>Anagallis arvensis</i>	91	44	48.4	10
<i>Medicago lupulina</i>	231	75	32.5	10
<i>Polygonum aviculare</i>	323	18	5.6	10
<i>Arenaria serpyllifolia</i>	437	68	15.6	9
<i>Bartsia odontites</i>	39	14	35.9	9
<i>Euphorbia exigua</i>	133	23	39.8	9
Grass spp.	44	2	4.5	8
<i>Papaver</i> spp.	39,389	1,182	3.0	8
<i>Alchemilla arvensis</i>	3,979	50	1.3	7
<i>Capsella bursa pastoris</i>	250	14	5.6	7
<i>Polygonum convolvulus</i>	19	2	10.5	7
<i>Alopecurus agrestis</i>	4,998	1	0.02	5
<i>Atriplex patula</i>	259	18	6.9	5
<i>Galium tricornis</i>	17	3	17.6	5
<i>Scandix pecten</i>	222	3	1.4	5
<i>Stellaria media</i>	49	1	2.0	5
<i>Veronica arvensis</i>	2,202	40	1.8	5
<i>Caucalis arvensis</i>	130	5	3.8	4
<i>Myosotis arvensis</i>	324	4	1.2	4
<i>Sonchus arvensis</i>	81	2	2.5	4
		<hr/> 1,610		

Achutha, *Anagallis* and *Medicago* have appeared steadily up to date, showing that they can have a period of natural dormancy of at least ten years. *Polygonum aviculare* produced a single seedling in 1935, after an interval of four years. This is noted for its prolonged dormancy, as it was the most abundant species which appeared on Geescroft Field at Rothamsted on land which had been under grass for thirty years (Brenchley, 1918). The remaining species show a maximum dormant period of four to nine years, though it is quite possible that some viable seeds are still present.

Most of the species retaining their vitality longest are those in which the largest *proportion* show prolonged dormancy, irrespective of the *number* of seedlings produced. The only exceptions are *Polygonum aviculare*, which experience has shown to be most erratic in its germination, and *P. convolvulus* and *Galium tricornis*, of which so few seeds occurred altogether that a single belated seedling represented a large increase in the percentage in the proportion surviving for more than three years after sampling. The high proportion of prolonged dormancy in the first two groups (Table VIII, excluding *Polygonum aviculare*) is noteworthy, and may afford some explanation of the abrupt seasonal changes in the abundance of the species, as the population in any one year is not necessarily dependent upon successful seeding in the

immediately preceding year, but may result from long-buried seeds becoming capable of germination and causing an unexpected influx of a particular species.

Under field conditions where fallowing is carried on for a limited time and the soil is also disturbed less regularly, the proportion of viable seed that reaches the surface in a given time may be less than in the shallow pans used for germination. Consequently many buried seeds which have passed out of their period of natural dormancy may perforce remain in a condition of induced dormancy, ready to spring up among the crops at a later date when cultivation eventually brings them to the surface. Species which produce many seeds that remain naturally dormant over long periods form an incalculable element in the weed flora. It is impossible to forecast their behaviour, for periods of great abundance of a particular species may be followed by times of great scarcity or even absence, after which it may again reappear in greater or less amount. The factors determining this behaviour are unknown and render such temperamental weeds, of which *Polygonum aviculare* is an excellent example, peculiarly difficult to deal with under field conditions. After eleven years' work it is now feasible to confirm or modify the opinions expressed in an earlier paper (Brenchley and Warington, 1930, pp. 246-60) with regard to periodicity of germination and the length of possible dormancy in the weed species concerned. In most cases the original suggestions have been to a large extent corroborated by the later work. The majority of species which showed steady decrease in the numbers germinating annually in the first set of samples behaved similarly in the later sets, while those showing irregular periodicity usually repeated this behaviour, though the direction of irregularity frequently varied. With *P. aviculare* the extreme irregularity of germination was confirmed, but the suggestion of a possible two-year period of natural dormancy was not borne out, as a certain proportion survived for ten years. The variation in behaviour of the two species of *Galium* proved constant. *Galium aparine* repeatedly behaved normally with a steady annual decrease in germination in each sample, and a period of dormancy not exceeding three years, whereas *G. tricornis* was irregular in periodicity, and showed a relatively large proportion of seeds with longer dormancy, up to a five years limit to date. *Aethusa* and *Euphorbia* confirmed the forecast, both being of very irregular germination and coming high in the scale of dormancy, both as regards time and number.

Some species which occasionally germinate in a normal, regular manner may usually be irregular in their behaviour, as *Anagallis*, *Atriplex* and *Medicago*, all of which were regular in 1925 samples, but generally irregular since. With *Anagallis* and *Atriplex* the suggestions have been confirmed, *Anagallis* giving a high proportion of seeds remaining dormant over a long period, while *Atriplex* has a relatively short dormancy.

Medicago is a most temperamental weed, and has entirely failed to come up to expectations. Instead of having a short dormancy as originally suggested,

it has proved to have one of the longest, with a very high proportion of seeds germinating after the third year. In fact, *Medicago* with 32.5 per cent. and a ten-year period is second only to *Anagallis* with 48.4 per cent. and a similar ten-year period. On the other hand, on some occasions, as 1932, a large proportion of the seeds were in such a hurry to germinate that the seedlings appeared thickly in the pans within two days of the samples being washed out and set up. A similar behaviour is sometimes noticed in the field on a wet stubble, when the ground may be carpeted with *Medicago* seedlings long before the land

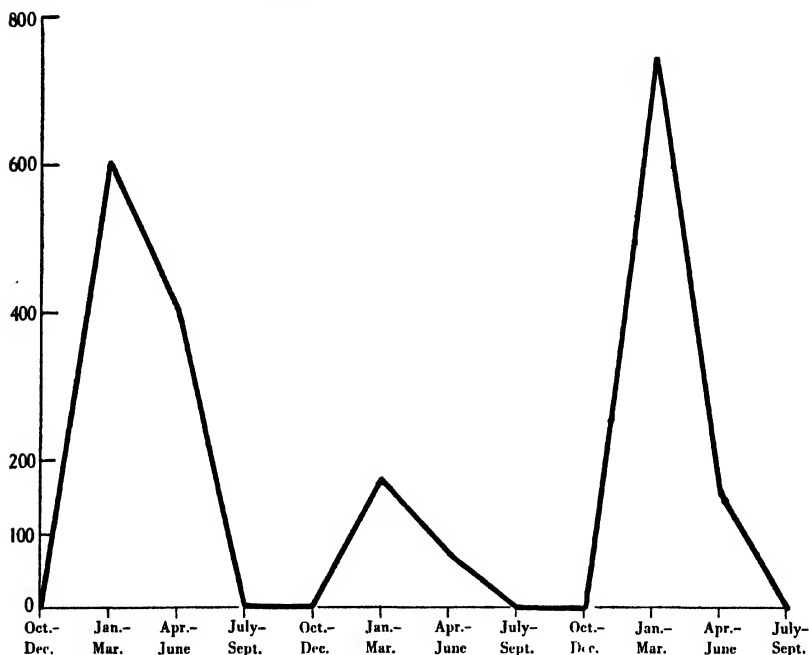


FIG. 8. Total number of seedlings of *Bartsia odontites* from all samples taken from 1925 to 1935, graphed according to season of germination.

is ploughed. The conditions which cause this behaviour are not clear. It is not merely a question of an abundant water supply, as, if it were, this premature germination should occur every year in the sample pans, whereas it is rarely seen there. It is probably some combination of climatic conditions during the ripening of the seeds which brings them to such a state of maturity that they are ready to germinate immediately an adequate water supply is available, no further period of after-ripening being necessary.

Caucalis has continued to show regularity in germination, but the indicated long dormancy has not been confirmed. On the contrary, the proportion of seeds lasting for four years has been quite low, and no later records have appeared. This result is unexpected and somewhat surprising, as in the field

Considering all species together, the number with three cotyledons was very low, only 136 out of a total of 641,171 on Broadbalk field, showing a ratio of 1 : 4714, confined to seven species of which two belonged to the genus *Veronica*.

In this connection reference may be made to *Spergula arvensis*, the dominant weed in a parallel experiment on sandy soil from Woburn, where five seedlings out of a total of 35,430 showed tricotyledony, giving a proportion of 1 : 7086.

VI. SUMMARY

1. Delay in cultivation after harvest prevents the reduction of various weeds by fallowing. Some species, already developed, continue to ripen seeds, and others have time to germinate and reach the seeding stage before they are cut down by cultivation. The numbers of extra seeds thus produced may be greater than those destroyed by fallowing, so that the reducing effect of the operation is entirely nullified.

2. When land is recropped after fallowing the first wheat crop tends to be abnormally heavy, thus introducing increased competition with the weeds. Some species fail to reassert themselves, but others are able to withstand the competition of the wheat and can replenish their stores of seed in the soil by the time the first crop is harvested. A few species vary in their response, either remaining at a low level or re-establishing themselves quickly after fallowing.

3. Species which soon begin to reassert themselves tend to increase rapidly in number and after three years under crop they may be much more plentiful than they were before fallowing began, *Alopecurus agrestis* and *Stellaria media* being notable examples. Other species tend to remain at a low level, and the varying rate of re-establishment results in a definite change in the balance of the weed flora. Though *Papaver rhoeas* was only reduced to about half its original number by fallowing, it has failed to increase to any great extent, so that it is no longer the dominant feature that it was before fallowing was begun.

4. Prolonged fallowing, for four years, reduces the store of buried weed seeds more drastically, but does not eliminate them all. The ultimate re-establishment of species follows the same lines as after a shorter period of fallow, though it takes longer for any species to regain its original numbers of viable seeds in the soil.

5. The period of natural dormancy of most species on Broadbalk proved to range from four to nine years, but four species may prove to be able to lie dormant for more than ten years.

6. *Bartsia odontites* showed very strongly marked periodicity of germination, as every seedling appeared between February and June, the majority appearing early in the year.

7. Relatively few abnormal seedlings have been observed in over 600,000 which germinated. A few albinos occurred in *Alopecurus agrestis* and *Papaver rhoeas*, and also a certain number of tricotyledonous seedlings belonging to seven species, chiefly *Papaver* spp., *Alchemilla arvensis* and *Veronica hederæfolia*.

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THE ESSENTIAL NATURE OF CERTAIN MINOR ELEMENTS FOR PLANT NUTRITION

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Until about the beginning of this century, ten or eleven elements only were generally considered as essential for normal growth of plants. The presence of other elements in minute quantities was recognized in many plants, but their possible association with nutrition and growth was not understood. The activities of the French investigators from 1897 onwards focussed attention on the function of these minimal traces of elements, and the work of Bertrand (9) on manganese, Javillier (70) on zinc and Agulhon (1) on boron may be regarded as the foundation of the widespread investigations which are now of such practical economic importance.

The French school claimed that some of these minor elements were essential for the full development of certain plants, but the experimental difficulties in obtaining adequate proof were not at that time fully overcome, and for a time the matter remained of academic, rather than practical, importance. Still, interest had been awakened, and much experimental work resulted in many and varied claims being made for different elements—claims which frequently were not borne out by other tests under different conditions. In 1923, however, Warington (160) was able to prove conclusively that a trace of boron is absolutely essential for the development of *Vicia faba*, as in its absence the meristematic tissues die and growth is impossible. Since then, investigations all over the world indicate that boron is probably essential for all plants in varying degree and that certain obscure plant diseases may be due to a deficiency of this element.

Although it is more difficult to get clear-cut proof of the essential nature of manganese for all plants, the whole body of evidence

is now so strong that this statement is generally accepted as fact. The small amount that is necessary and the closeness of the association of manganese with iron render this element peculiarly difficult for experimental treatment. Copper and zinc have also attracted much attention and their value in certain cases seems evident, though they cannot lay claim to the importance of manganese and boron.

Work has also been done with a wide range of other elements, often with conflicting results and in no case affording proof of their universal value. Stimulation of growth is frequently recorded, but up to the present most of the information gained deals with the relative toxicity of larger amounts of the elements. It must not be forgotten, however, that there is a possibility that certain elements may prove to be essential for certain plants, though not for all. If this is the case, the proof of the association of the particular element and plant will be difficult to obtain by deliberate investigation, but is more likely to be the result of a fortunate chance observation.

During the last thirty-five years the literature on the relation of minor elements to plants has become very extensive. Willis' (165) bibliography covers between 2000 and 3000 references with abstracts, and Jacks and Scherbatoff (69) give more than 350 others, but even so, the whole field is not covered. Much of this literature deals with the toxic and fungicidal aspects of the subject, but in the present review attention is chiefly confined to work done on the possible essential nature of certain elements for plant growth during the last five years. In spite of this narrowing of the subject, it is almost invidious to select certain papers for comment and to omit others, and such omission in no way implies that any particular piece of work is of less value than those actually quoted. Anyone who is interested in any particular element is strongly recommended to refer to the two bibliographies above mentioned in order to get a more adequate idea of the available information.

BORON

The essential nature of boron for many species of plants was fully established before 1930, and the possible connection of boron deficiency with certain plant diseases was already under consideration, as in the extensive investigations of Mes on "toppsickte" in

tobacco (102). During the last five years the practical aspect of the matter has been widely investigated, and boron deficiency is now suspected to be the cause of a variety of obscure physiological diseases which cannot be traced to insect or fungus attack.

Heart-rot, crown-rot, or dry-rot of sugar beet is characterized by the blackening and death of the central leaves, coupled with discoloration or rotting of the upper part of the root. Late in the season secondary growing points develop numerous small leaves, giving the plants a very dense, short, green top. Where the disease is prevalent, the yield is often very low and the sugar content considerably decreased (137). It has been found repeatedly that the application of small quantities of boric acid or borax to the soil effectively prevents or cures the trouble (18, 21, 49, 63, 74, 141). The necessary quantities range from about $4\frac{1}{2}$ to 9 pounds of boric acid, or 10 to 20 pounds of borax per acre, the best time of application being at or before sowing (16, 17, 43, 106). Where the disease occurs on mildly acid soils it is associated with lack of boron compounds, but it is also found on alkaline soils which contain as much boron as should suffice for normal growth. In the latter case, it seems probable that the boron is in some way locked up and rendered unavailable for the plants. This hypothesis is strengthened by the increasing amount of heart-rot that is being found in districts where the soil is limed, pointing to a gradual withdrawal of the boron from its available condition. In England and Scotland the disease is only gradually being recognized and reported, but an increase of the trouble is anticipated if heavy liming of the sugar beet areas is carried out, as the danger of excessive calcium is already realized (13). The association of heart-rot with boron deficiency has been confirmed in controlled water culture experiments, in which similar symptoms can be induced. It is also claimed (8) that the presence of boron increases the resistance of sugar beet to poisoning by the heavy metals. Solunsky (153) has examined the growth conditions which determine the effect of boron deficiency on sugar beet and suggests that water relations are of great importance. His conclusions are that the increase of moisture in the soil during the first half of the vegetative period stimulates the development of foliage and aggravates the disease, while at the very end of the vegetative period, in some cases, it may result in recovery of the plant. Fron (44) also suggests that

one of the chief factors causing heart-rot is lack of water caused by the drying out of the soil.

Brown-heart of turnips is a parallel disease which has been successfully controlled in Canada (89, 90) by the application of ten pounds per acre of borax. This has since been confirmed by experiments in Scotland and Wales (114a, 169).

Boron is now generally recognized as essential for tobacco, deficiency producing characteristic symptoms of disease (152). As generally happens, the meristematic tissues are primarily affected, the stem apices die and flowering is inhibited, the effect on roots and leaves being less marked. Diseased leaves are richer in starch and sugar than healthy ones, possibly because the disorganized phloem interferes with normal transport (146, 147). Deficiency of calcium resembles that of boron in that both produce death of the terminal bud, but calcium shortage shows first at the tips of the young leaves, whereas boron deficiency is first seen as a light green color at the base of the young leaves, followed by a general breakdown (91, 93). Although the evidence is not conclusive, some indications of an association between the absorption of boron and that of calcium have been obtained in *Vicia faba* (162).

Mes (103) states that the symptoms of deficiency are most marked when vegetative growth is strongest, as has repeatedly been found with *Vicia faba* at Rothamsted. Attempts to replace boron by manganese were ineffective, though the manganese improved the vegetative growth and the green color. Boric acid or borax in small quantities has proved effective in ameliorating this deficiency, 5 pounds per acre being adequate in some cases (94). In Sumatra for years past boron compounds have been used as fertilizers in the ordinary commercial routine, their value as a preventive of disease being fully accepted (79, 104).

The tomato, another member of the Solanaceae, also needs boron for normal growth and for the setting and development of fruit (71, 151). The boron is apparently fixed in the tissues and cannot be used repeatedly by the plant, so a constant, though minute, supply is essential throughout the period of growth. When the main growing points are killed by deficiency, the buds are stimulated to develop till the boron supply is locally exhausted, when symptoms of degeneration again appear (146, 147).

Evidence for boron requirement of cereals is less definite, pos-

sibly because the minimum requisite amount is very low, enabling normal growth to be made under conditions which would induce disease in many other species which require larger amounts (138). The range of boron content in various species has been found to be lowest in barley, rye and wheat, from .1 to .3 mg. per kgm. dry weight, and highest in tomato, tobacco, potato, beans and peas, rising to a maximum of 18 mg. per kgm. dry weight (157).

Boron deficiency is accompanied by abnormal tillering in wheat, which can develop right up to the flowering stage in solutions containing no boron (111). The ears, however, either do not emerge or are badly developed and sterile, a condition which also appears in maize under similar circumstances (122). Oats may possibly need boron, though this is not fully established, as the yield of straw has been increased by boron manuring, but the yield of grain reduced (88). The incidence of fungus disease appears to be influenced by boron supply, and Eaton (38) found that *Erysiphe graminis* was abundant on boron deficient barley plants when it was absent from those receiving boron, whereas *Helminthosporium sativum* behaved inversely, the attack increasing in severity with increasing amounts of boron in the nutrient solution.

The injurious action of boron for citrus trees is widely recognized, and sufficient is contained in some irrigation waters to damage both citrus and walnut (143). Minute quantities are, however, essential and the anatomical and physiological changes induced by boron deficiency have been reproduced in controlled experiments (50, 53, 54). Here again the meristematic tissues are primarily affected, gum formation following. Abnormal carbohydrate accumulation occurs in the leaves, but as this excess is rapidly reduced if boron is supplied, it seems probable that an improvement in the conducting tissues plays an important part in the recovery of the plant.

Symptoms of boron deficiency manifest themselves later and develop more slowly during spring and autumn than in summer. This appears to be due to the reduced length of day (161), rather than to lower temperature. A certain correlation exists between the factors of boron and length of day as, with a variety of species, in the absence of boron the influence of length of day was found to be less striking than when boron was present, whereas

the boron deficiency symptoms were less pronounced under short day than under full day conditions.

Now that the signs of boron deficiency are becoming known, growers are beginning to attribute various cases of unhealthy or failing crops to this cause, and in many cases the trouble can be overcome by the use of small dressings of boron compounds. Sugar cane in water culture experiments exhibited depressed growth, distorted and chlorotic young leaves, and definite stem and leaf lesions in the absence of boron, normal growth being resumed if as little as .22 p. p. m.¹ of boron was added to the nutrient solution (99). The demands of strawberry are somewhat greater, varying according to the season of the year, 1 p. p. m. of boron preventing deficiency symptoms in spring, but not in summer. Cases have been observed in field conditions where deficiency of boron has definitely limited growth (57). Flax soon perished without boron, showing decay of the growing points of the shoots, and bad development of lateral rootlets (148). In water cultures the dry weights obtained were

With boron	8.07 gm.	100	per cent
Without boron14 gm.	1.73	per cent

Under soil conditions, better growth occurred if boron was added, and the flower buds appeared earlier. Overtreatment with calcium- or manganese-carbonate on certain soils causes boron deficiency in flax, as it does with sugar beet, a condition that can be remedied by fertilizing with boron compounds (156).

Claims have also been made for the need of cotton (39), red clover (47), soybean (114), lettuce (86, 87), buckwheat (105) and blueberry (145) for boron, and doubtless other species will be added to the list as time goes on. Germination of maize and the early stages of growth in potatoes have also shown benefit from traces of boron (134). Care will be needed, though, not to attribute all obscure plant diseases to boron deficiency without adequate proof. Although it is perfectly clear that in many cases it is genuine damage due to lack of boron which is cured by the application of boron, we do not yet know whether in the soil there are other conditions causing unhealthiness in plants which are remedied

¹ Parts per million.

by treatment with boron. If this should be the case, the benefit of boron would be indirect, and not direct, but so far no evidence of this type of action has come to light, except in the possible case of rubidium injury in potatoes, which has been mitigated by the supply of available manganese and boron (56).

COPPER

For many years past the importance of copper has been recognized in connection with its function as the active principle of Bordeaux mixture, used for controlling fungal disease on important cultivated crops, as *Phytophthora infestans* on potato and *Peronospora viticola* on vines. This naturally led to enquiries as to the possible harmful action of the copper which falls to the ground during spraying, but all evidence in this respect has been negative, as the copper forms insoluble compounds in the soil with chalk, oxide of iron and alumina, and is, therefore, removed from the sphere of action (128).

More recently, the importance of copper in certain aspects of animal physiology has attracted much attention, and suggestions have been made that this element, in very minute quantities, may also be essential to plant life. This claim is by no means proved, but there are definite instances where copper has certainly improved growth in one way or another, though it cannot yet be regarded as "essential" in the true sense of the word. Far more critical work is necessary before copper can be considered on the same footing as boron and manganese.

The most spectacular work with copper was that of Allison, Bryan and Hunter (3) in 1927 on the sawgrass peat in Florida, when they found that the wholly unproductive soil could be made to produce excellent crops of lettuce, radish, turnips, rape, tomatoes, etc., by the addition of 30 pounds of copper sulphate per acre without any further manuring. Since then, various workers have found that copper is beneficial to crops grown on peat and muck soils (164), but, as in some cases similar effects can be obtained by the use of certain manures, as potash (167, 173), it is still a moot question as to whether the action of the copper is directly on the plant, or whether it acts indirectly by ameliorating some adverse condition in the soil. Allison (2) has attacked this problem and claims that in sugar cane normal development follows if copper is

introduced into the plant in other ways than by the roots, though this still does not eliminate the possibility of some action of copper on toxins absorbed from the soil. Whatever the explanation, the benefit itself cannot be questioned, and in Holland it is the recognized practice in reclamation of peat land to add 50 kg. per hectare of copper sulphate during the first year, as a preventive of what is known as "Urbarmachungskrankheit" or reclamation disease (cf. 20, 149, 171).

Certain types of chlorosis can be remedied by application of copper salts, either with the fertilizer at the roots or by spraying the leaves. The "frenching" or spotting of citrus leaves has been cured by spraying with Bordeaux mixture or by applying copper sulphate to the soil (117). Despite the increase in chlorophyll production, however, no copper was detected in the chlorophyll itself. Exanthema in pear trees is also attributed to copper deficiency, but there is no evidence as to whether the disease is due to the absence of copper *per se*, or to the presence of soil toxins, the effect of which is neutralized by the action of copper (118). Chlorosis of other deciduous fruit trees has also been cured by the use of $\frac{1}{2}$ -2 pounds copper sulphate per tree, applied to the soil (5, 67). From another point of view the addition of copper sulphate to the usual fertilizers is said to improve the thickness and color of onion scales, though the results are not always consistent (77, 78).

Increased yields due to treatment with copper have been claimed in various quarters (135). Oats (19, 88), tomatoes (151), maize, sweet potatoes and beans (130) are among those mentioned and indicate the wide range of plants that apparently respond to copper, though in other cases no benefit was found with tomatoes (119) or buckwheat (105). It must, however, be remembered that an element may stimulate growth without being essential in the sense that in its absence vital aspects of growth are inhibited or seriously checked. More proof than this is needed, and certain investigators have applied more critical methods. Using water culture methods, Lipmann and McKinney (81) found that flax grew satisfactorily without copper till blossoming, but the amount of bloom was reduced and no capsules or seeds were produced. Barley also needed copper for seed formation, 1/16-1/18 p. p. m. of Cu in the nutrient solution being sufficient. Sommer (154), on the other hand, states that flax, tomatoes and sunflowers made little growth without cop-

per after the first week in nutrient solution. None of these workers has any clue to the rôle of copper, though it has been suggested that it may be auto-oxidant or catalytic in action. Among lower plants copper, as well as manganese and zinc, has been found to increase the growth of *Aspergillus flavus* and *Rhizopus nigricans*, better results being obtained from a combination of these elements than from each separately (84). The fat content was also increased but the proportion of nitrogen decreased by a very low optimum amount, the toxic limit soon being reached.

Although the claim for the essential nature of copper in the economy of plants cannot yet be substantiated, the evidence in hand is sufficiently encouraging to justify more extended investigations under strictly controlled conditions, for it is quite possible that copper may be essential for some plants or under certain conditions, and be unnecessary in other cases and for other species.

MANGANESE

The recognized importance of manganese for growth has encouraged work on the manganese content of plants, which varies considerably between species and also in a single species grown under different conditions. Lundegårdh (82) states that manganese is very slowly absorbed and that the total soil manganese has no great influence on the uptake. The addition of manganese sulphate to the soil may increase the manganese content of crops grown thereon (28), and with spinach a larger amount was found to be absorbed when the fertilizer was applied in several small dressings instead of all at once in a single treatment (108). This may imply that the sulphate remains available only for a short time and that repeated small applications enable the plants to utilize a larger proportion before the remainder becomes unavailable. The manganese content of plants grown in soils of varying acidity has been found to increase with the hydrogen ion concentration of the soil, due to a higher proportion of manganese in the soil water of acid soils (116).

Grasses vary considerably in their ability to take up manganese from the soil, the average content in one test varying from 207.5 mg. per kilogram for *Dactylis glomerata* to 78.1 mg. per kg. dry matter for *Poa pratensis*, while lucerne was lower than any grass, with only 46.6 mg. of manganese per kilogram (14). It is gen-

erally found that the proportion of manganese is higher in the leaves than in other parts of vegetables and fruits (126). Also, for any one species at any time the green leaves are always richer in manganese than etiolated, chlorotic leaves (11). The demands of species vary considerably, as is indicated by variation in the response of different crops to manganese fertilizers.

From the practical point of view the importance of manganese lies in its ability to prevent chlorosis and to increase the yield of crops. Manganese deficiency disease is usually manifested by a loss of green color, and is apt to be most marked on soils of high pH (30), rarely showing itself on acid soils. Heavy liming on some soils is, therefore, often followed by the appearance of trouble (80) due to the manganese in the soil being rendered unavailable for the plant, and where such liming is necessary for the production of certain crops the use of manganese fertilizers is essential (166).

Chlorosis due to manganese deficiency shows itself in characteristic ways. In tomato and cucumber plants the tops are first affected, the intravascular tissue of the leaves gradually changing from green to yellow while the veins and midribs remain green, producing a definitely mottled appearance. The general growth is weakened and the flower buds usually turn yellow and fall before opening (59). It appears that certain factors, as low temperature and slow growth, enable plants to withstand manganese chlorosis and that the trouble can also be overcome either by the addition of manganese compounds to the soil or by the correction of the soil reaction so as to make the manganese which is present available to plants (46). This correction can be made by increasing the acidity by the application of sulphur or ammonium sulphate, or by causing temporary water logging, in which the high degree of water saturation acts as a reducing agent (123). Many crops are improved either in health or yield by the application of manganese fertilizers, including blue lupins, soybeans (97), oats (29, 88), spinach, beets, blueberry (145), buckwheat (105), tomatoes (68, 129) and cucumbers, 100-150 lbs. per acre of manganese sulphate being effective with the latter (58). When all the tests are reviewed, however, it is evident that crops differ considerably in respect of their manganese requirements (115). In some cases, the reports

indicate that no benefit has been derived from manuring with manganese (64).

On the other hand, a type of chlorosis is induced also by excess manganese, as may occur with blue lupins on limed soil if there is a deficiency of iron (142), while poor growth of rice has been observed in the presence of an excess of soluble manganese in the soil (72). Chapman (25) has put forward the hypothesis that excess of manganese hinders the transport of iron to and from the leaf by converting it into an insoluble ferric form, thus causing chlorosis.

Citrus, sugar cane and tobacco are three other crops which react to deficiency of manganese. In Florida and California the growth of citrus and the quality of the fruit have been much improved and any tendency to chlorosis overcome on alkaline soils by applications of manganese salts (150). Haas (51) found that in acute cases of deficiency, citrus leaves absciss prematurely and the shoots die back. The roots remain healthy after deficiency symptoms are manifest in the shoots, suggesting that the roots absorb what manganese is available and retain it, not surrendering it to the stem and leaves unless more manganese is supplied.

Sugar cane exhibits the usual type of chlorosis due to manganese deficiency, and the yield and quality of the sugar is also affected (34, 98). The purity of the cane sugar is usually higher on soils with high manganese content (158), and there is also less brown-stripe disease on such soils (55). Sugar beet also shows increase in yield and sugar content if manganese deficiency is eliminated (48). The relation of sugar to manganese has been worked out in other species. Wheat, maize, lettuce and tomatoes grown without manganese have been found to be lower in reducing sugar and sucrose than those receiving manganese, indicating that the element plays some important part in sugar formation and sugar metabolism. In these experiments the manganese was injected straight into the stems, thus enabling both control and manganese plants to be grown in the same pots under identical conditions of nutrition and environment (107).

Apart from chlorosis, various other observations with relation to manganese have recently been made. Tobacco, while showing the usual deficiency chlorosis (93), is also very sensitive to excess, as plants growing on acid soils may be injured by toxic quantities

of soluble manganese (15). In such cases, phosphate fertilizers reduce the injury, probably by rendering the excess manganese inactive in the plant. Walnut yellows is a disease that is still little understood and for which manganese deficiency has been suggested as the cause. As, however, affected walnut leaves and bark contain a higher proportion of manganese than the healthy tissues, the cause cannot be attributed to manganese deficiency unless a considerable amount of the element that is present is for some reason or other unavailable for use in plant metabolism (52).

A most important aspect of manganese deficiency is its relation to grey-speck disease of oats and wheat. Inspired by Samuel and Piper (132), various investigators have obtained control of the disease by judicious use of manganese (33, 101, 163). Gerretsen, however, is now claiming that this does not represent the whole of the story, but that other factors of a bacterial nature combine with the manganese deficiency to cause the disease (45). Manganese deficiency in barley has been dealt with effectively by drilling $\frac{1}{2}$ cwt. of manganese sulphate with the seed (144).

Claims have been made that small quantities of manganese stimulate the growth of various plants (136). As, however, this has been specifically claimed for plants grown under alkaline conditions it remains an open question as to whether a genuine stimulation occurs, or whether it is merely that an incipient manganese deficiency, causing reduction of yield without external symptoms of damage, is overcome by the application of manganese salts, resulting in an improvement in growth which suggests stimulation. Seeds of chickpea and peanut treated with .5 per cent solutions of manganese sulphate before sowing have been found to grow faster than control seeds for the first 10–20 days, this being attributed to the effect of the salt in accelerating enzyme activity during mobilization of food reserves and early stages of plant growth (172).

A certain amount of work has been continued with cryptogams and simpler phanerogams. Traces of manganese are beneficial to yeast, increasing the dry weight (85), though the toxic limit is soon reached, resulting in decreased growth or death of the cells. Further claims are made for the importance of manganese for *Aspergillus niger*, for which it appears to be essential for normal growth and sporulation (155). Hopkins (60, 61) found that the green alga *Chlorella* made no growth without manganese and sug-

gests that the element functions physiologically in an indirect manner by its action on the state of oxidation of iron, so that sufficient manganese must be present to ensure the oxidation. The evidence in regard to *Lemna* is conflicting, as workers who originally stated that manganese had no beneficial effect (27), have repeated their work and now state that minute traces in the nutrient salts must have been overlooked, and that it appears that *Lemna major* does need a trace, though 1 : 300,000,000 is sufficient to give good growth (26). Here again the toxic limit is very soon reached, 1 mg. Mn per litre being too high a concentration for optimum growth (131).

ZINC

The essential nature of zinc in fungus nutrition has been claimed for many years, since Raulin (125) worked in 1869 with *Aspergillus niger*. After much controversy, the general opinion is that this view is correct, and various workers have put forward further proof in the last five years. Steinberg (155) states that normal growth and sporulation of *Aspergillus niger* can occur only in the presence of several minor elements, including iron, copper, manganese and zinc. The dry weight of yeast (85) is also increased by zinc, though, as usual, too heavy doses are toxic.

Among the higher plants claims are made for the value of zinc from two aspects—as a stimulant to the growth of certain crops, and as a specific against certain diseases. McHargue and Shedd (88) obtained increases in straw and grain of oats by the addition of traces of zinc to sand cultures, whereas Scharrer and Schropp (140) found greater stimulation with other cereals and peas than with oats. Buckwheat and flax have also been improved by zinc in some circumstances as, for instance, where flax was grown on heavily limed acid soil (73).

The more important aspect is in relation to plant diseases which are attributed to a deficient supply of zinc. Frenching or mottle-leaf of citrus, little-leaf of fruit trees (96), court-noué of vines (36), pecan rosette (42) and bronzing of tung trees (113) have all been successfully treated by zinc sulphate, leading to the assumption that zinc is essential for certain metabolic functions and that a deficiency of the element hinders normal development. Applications of zinc sulphate to the soil, varying from .25 to 15 lbs. per tree, according to the type of citrus, have caused marked im-

provement in badly frenched trees (23), but this method sometimes fails. Spraying with solutions of zinc sulphate is more generally successful (159) and sometimes direct injection into the tissues is satisfactory (95). According to Dufrenoy and Reed (37) zinc, as well as iron, has a specific effect on leaf assimilation, mottle-leaf being a pathological symptom indicating an interruption of equilibrium between the cytoplasm and its inclusions. The provision of zinc to affected plants increases chlorophyll production and photosynthetic activity. A significant point is that zinc is present in the cells of treated, but not untreated, orange trees, leading to the direct association of zinc with recovery of the plant.

Little-leaf or rosette of fruit trees is characterized by the production of numbers of abnormally small leaves, and the value of zinc as a corrective is acknowledged from many quarters. Opinions differ as to the best method of application, and soil applications, injection into the tissues (35) and spraying (121) or dipping all have their advocates. While it is probable that little-leaf of fruit trees is a symptom of an inadequate supply of zinc for normal metabolism, Chandler, Hoagland and Hibberd (24) point out that the trouble may not be due to zinc deficiency only, as large woody perennials grown on the same soil as the fruit trees are also susceptible, while annual plants are generally free from attack. They suggest the possibility that zinc may aid in the precipitation of toxic substances formed by certain soil bacteria, and that the beneficial action of zinc may thus be indirect rather than direct.

Though the beneficial effect of zinc in these various types of abnormal development cannot be denied, no definite proof yet exists that zinc is essential for normal development of higher plants. Such proof can be given only by experiments in which plants are grown from seed in the entire absence of zinc, as has been done with boron, copper and manganese. If little-leaf, frenching, etc., could then be produced artificially, the practical results already available would provide a most valuable weight of evidence in support of the hypothesis.

OTHER ELEMENTS

Up to the present, definite evidence of the essential nature of "minor" elements has been established only for the four elements already examined. A considerable amount of work has been done

with others with the same objective, but so far with very little success. The much-tested fungus, *Aspergillus niger*, has shown some stimulation with very low strengths of various elements (124), higher concentration being very toxic. Other elements exhibit toxicity and among the halogens fluorine is the most poisonous. With higher plants only indifferent or toxic action has so far been proved with such elements as bromine (168), fluorine (105, 112, 133), molybdenum (139), selenium (65, 66, 76), uranium (7), thallium (62, 83, 92), caesium (4), palladium, beryllium and zirconium (6, 22). Rubidium is usually found to be toxic or indifferent (4, 22) though Loew had earlier claimed that in small amounts it benefited Chinese cabbage, barley and spinach. Rather more evidence of occasional stimulation occurs with a few other elements, described below in more detail.

ALUMINUM

Toxicity of aluminum is repeatedly being shown (41) but there is no evidence that small quantities of the element are essential for growth. The beneficial effects occasionally recorded are generally due to soil reaction caused by the aluminum liberating supplies of definitely necessary elements, such as iron (127), of which scarcity causes chlorosis.

ARSENIC

Arsenic is also noted for its toxicity (31, 120), but in conjunction with other minor elements it has been found to increase the frost resistance of young maize plants under certain cultural conditions (122).

BARIUM, LITHIUM, STRONTIUM AND CHROMIUM

The first three may possibly play some part in the metabolism of sugar cane, as they have all been found in very productive soils, while they were absent in poor soils. Strontium, with chromium and zinc, may also exercise some inhibitory action on diseases of sugar cane, which is interesting in view of the stimulation exercised by strontium on *Aspergillus niger* in high concentrations (124). In some areas, the proportion of brown-stripe disease of sugar cane varies inversely with the amount of chromium present in the soil (55).

CADMIUM

It has been claimed that cadmium stimulates oats, rye, wheat and barley grown in sand or water cultures, the concentration varying with the species, but maize was not found to respond. The degree of stimulation was less than that obtained with equivalent amounts of zinc (140). Low concentrations of cadmium have also been found to stimulate growth of *Aspergillus niger*, high strengths being toxic (124).

COBALT

Cobalt is a widely distributed constituent of plants, occurring in small quantities in many species. No relation has yet been proved (12) between plant growth and the presence of cobalt, though some workers have suggested that both cobalt and nickel may act as catalyzers in the living plant (10). Cobalt in great dilution has been found to act favorably on *Aspergillus*, though the effect is not so clear as with nickel (110).

IODINE

While it is generally recognized that iodine is toxic in stronger concentrations (109, 170), opinions differ as to its action in great dilution. Whereas Meyer claimed that iodine is essential for the best growth of buckwheat (105), Cotton states that it exerts no beneficial action on that species even in great dilution (32). Potassium iodide has been found to improve lettuce, cucumber and tomatoes, causing quicker growth and preventing crimping in lettuces and stem-rotting in tomatoes (75).

MERCURY

Advantage is taken of the toxicity of mercury to control such diseases as potato-scab, at the risk of reduction of crop (40, 90). The possibility also exists that mercury may precipitate the toxins causing little-leaf of fruit trees (24), but no directly beneficial effect upon growth has been shown.

NICKEL

Nickel is so widely distributed that it may almost be regarded as a normal constituent of plant tissues (100), but it has not yet been proved that it plays any essential part.

TUNGSTEN

Stimulation of the early growth of seedlings of various cereals and peas has been observed with low concentrations of tungsten in the form of sodium tungstate, the beneficial strength varying considerably with the species (139).

SUMMARY

The available evidence makes it quite clear that small amounts of boron and manganese are essential to the growth and health of many, if not all, species of plants. Copper and zinc have also been found to be necessary in many cases, though up to the present it is uncertain whether this need is universal. Apart from these four elements, isolated cases only of improvement due to traces of other minor elements have as yet been established. It may be, however, that specific elements are necessary for specific plants, and it is possible that the conclusive evidence already obtained with boron and manganese may further the opening of a wide field of investigation which may lead to results of far-reaching importance from scientific and economic standpoints.

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A Study of Heart-rot of Young Sugar-beet Plants Grown in Culture Solutions.

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With eleven Figures in the Text.

INTRODUCTION.

WARINGTON (10) has reported on the changes in anatomical structure induced in broad beans by growing them in a medium free from boron. She found that wilting and ultimate death of the plants was preceded by basipetal degeneration of the tissues from the apex, which was at first confined to the cambium and vascular tissues of the plant. In *Vicia faba* Warington found that, if the plant was not irreparably damaged, the addition of boron to the nutrient solution caused the development of new axillary shoots at the base of the main shoot.

It has been known for some time that the disease called 'heart-rot' in sugar-beet is due to boron-deficiency. This fact has been demonstrated by Brandenburg (2, 3), de Haan (4), Kaufmann (7), Foex and Burgevin (6), Bobko and Belousov (1), &c. In view of the abnormal type of secondary thickening in Beta it was thought that a study of the anatomy of boron-starved plants might prove interesting since, normally, separate concentric cambia are cut off in the ground tissue at intervals throughout the life of the plant. It seemed possible that the death of one cambium need not necessarily preclude further growth in thickness in the same organ when boron was restored to the nutrient solution. Thus, zones of normal and degenerating tissue might be obtained in the same organ, and an opportunity would be provided for observing stages in disintegration.

Reference to the literature made it clear that sugar-beet is very sensitive to boron in great dilution, and that just as in broad bean, while a concentration of 1 : 1,000,000 boric acid is enough for normal development, in the absence of boron growth is inhibited and disintegration sets in. Accordingly, a number of sugar-beet seedlings were raised and transferred to culture solutions.

EXPERIMENTAL METHODS.

Fruits of sugar-beet were set in damp sawdust and seedlings were available in about nine days. Forty-two of these were transferred to covered bottles containing nutrient solution of the following constitution :

KNO ₃	1 g.	KH ₂ PO ₄	0.3 g.
MgSO ₄	0.5 g.	K ₂ HPO ₄	0.27 g.
CaSO ₄	0.5 g.	Fe ₂ Cl ₆	0.04 g.
NaCl	0.5 g.	MnSO ₄	0.001 g.

Water to make up to one litre.

This is one of the Rothamsted food solutions, and its pH is about 6.3. The chemicals used had previously been tested by spectroscopic methods, and were guaranteed to be free from all traces of boron.

The experiment was begun in the second week in May 1935, and terminated by fixation of the material on 6th August of the same year.

PLAN OF THE EXPERIMENT. TABLE I.

(Seeds set in damp sawdust on 9th May 1935.)

Set.	16th May.	5th June.	26th June.	15th July.	31st July.	6th Aug.
A	-B	B	B	B	-B	Fixed
B	-B	2B	2B	2B	-B	"
C	-B	-B	-B		dead	
D	B	B	-B	+L	B	Fixed
E	B	B	B	-B	-B	"
F	B	2B	2B	4B	8B	"

As a fixative, 2BE (LaCour (8)) was used, and this gave good results. After sectioning the material, it was found that staining with gentian violet alone was more satisfactory than the use of a double stain.

The plants were divided into six sets of seven each (see Table I).

I. *Plants grown without boron for the first three weeks.*

A. Transferred to a solution containing 1 p.p.m. H₃BO₃ on 5th June, and deprived of boron again one week before fixation.

These plants were greatly retarded at the end of the first three weeks, most of them having small root-systems and no expanded leaves except for the cotyledons. After the transference to boron, however, the growth-rate rose considerably, although at six weeks old the hypocotyls were not yet swollen, and the leaves were still very small as compared with the sets that from the outset had been supplied with boron. Recovery continued in all except one plant, which succumbed in the tenth week. The rest of the set had several expanding leaves and swollen hypocotyls by the end of July. On 31st July they were replaced in boron-free solutions, but no signs

of heart-rot, either external or internal, had set in when they were fixed on 6th August.

B. Transferred to a solution containing 2 p.p.m. H_3BO_3 on 5th June, and deprived of boron again one week before fixation.

Recovery after the initial three weeks deprivation of boron was more rapid in this set than in that receiving 1 p.p.m. of boric acid (the A set), and none of the plants died. By the middle of July all of these were sturdy plants with swollen hypocotyls, and growth in boron-free solutions for a week before fixation produced no sign of heart-rot.

In both the A and the B sets the original terminal bud had died, and growth was continued by two or three closely-set axillary buds at the top of the beets.

C. Maintained in boron-free solutions.

These plants did not grow beyond the seedling stage, and by 28th June they were all dead.

II. Plants grown with 1 p.p.m. boric acid for the first three weeks.

D. Transferred to fresh solutions containing boron for three weeks, deprived of boron on 26th June for a further three weeks, then supplied with boron until the end of the experiment three weeks later.

These plants grew well, and had a number of leaves and thickened hypocotyls by 26th June. A fortnight after this all of these plants showed unmistakable signs of the disease. The older leaves were quite healthy, but the bases of the younger leaves and the apical buds were black and shrivelled. With the restoration of boron on 15th July these plants began to recover. By 31st July, all the blackened leaves had dried up, and new green leaves from axillary buds and new roots were developing rapidly, progress continuing steadily until the end of the experiment. The plants appeared rather bunchy owing to the large number of small leaves growing out from the top of the hypocotyl. On cutting the swollen beets open longitudinally, it was seen that they were all more or less marked with brown streaks.

E. Transferred to fresh solutions containing 1 p.p.m. H_3BO_3 for another six weeks, deprived of boron on 15th July, and kept without boron until the end of the experiment.

By 15th July these beets were considerably swollen, and each had a number of well-grown leaves. After a fortnight in boron-free solutions blackening of the apical buds and the bases of the younger leaves had taken place, while the older leaves remained quite healthy in appearance. This condition became accentuated during the last week of the experiment,

gradually including older leaves as the disease spread outwards. The beets were split open longitudinally, and were all found to be more or less marked with brown streaks at the top, which were particularly evident in one plant.

F. Given 2 p.p.m. H_3BO_3 on 5th June, 4 p.p.m. on 15th July, and 8 p.p.m. on 31st July.

Throughout the experiment, these plants were healthy and actively growing. After six weeks in solutions containing 2 p.p.m. H_3BO_3 , they showed no improvement on the last (E) set that had, up to that time, been supplied with 1 p.p.m. The higher concentrations given later on had no apparent adverse effect on the metabolism, as it was clear that the plants had not been poisoned by a concentration of 8 p.p.m. H_3BO_3 .

Considerable resistance to the absence of boron in the nutrient solution was shown by the young seedlings of sets A, B, and C, and this was presumably due to the presence of this element in the cotyledons. Before the end of three weeks this reserve was exhausted, and doubtless the plants would not have recovered if the addition of boron had been delayed much longer. The plants were only thirteen weeks old when they were fixed, and through the whole of this time boron appeared to be of definite importance in the metabolism of this plant, since at all stages, after that of the young seedlings, signs of disintegration were visible in ten days after the change-over to a boron-free solution, although not in less time than this.

STRUCTURAL CHANGES DUE TO ABSENCE OF BORON.

I. *General outline.*

A study of serial sections of a beet reveals the fact that each of the rings of secondary vascular tissue, to the development of which the swelling of the beet is due, is composed of downward continuations of leaf-traces. These vascular rings are considered by Eames and MacDaniels (5) to originate in the proliferating pericycle. The rings are not regular, but consist of two or more arcs, each representing a single leaf-trace. Probably the number of arcs in each ring depends on the phyllotaxis of the leaves to which they are connected.

Sets A and B.

(Grown without boron for three weeks, then given boron till a week before fixation).

In these plants, as a result of the early destruction of the original growing point due to a lack of boron, two or more buds have grown out, each giving rise to its own set of vascular rings. The diseased vascular system has been pushed over to one side, and appears in transverse view near the top of a longitudinal section of the beet (Fig. 1).

Set D.

(Grown in the presence of boron except for three weeks during June and July).

In this set of beet the central vascular ring is without any sign of cellular disintegration. The next few rings are normally developed, but

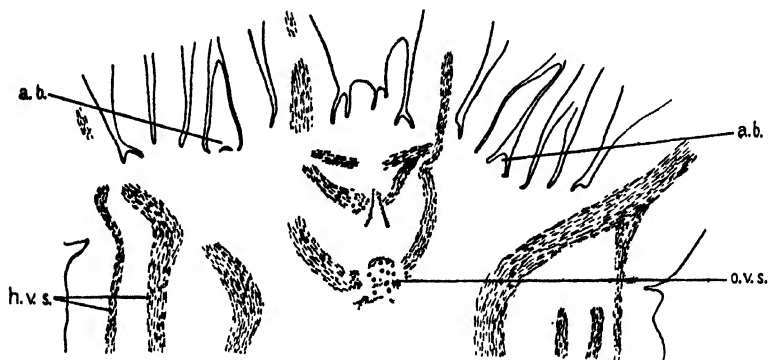


FIG. 1. Nearly median longitudinal section of a beet from the A set (grown without boron for three weeks, then given boron until a week before fixation). *o. v. s.* original vascular system of the seedling cut transversely. Diseased cells are dotted. The scattered cells represented are vessels. *h. v. s.* healthy vascular strands formed by the leaves which were developed on recovery of the plant. *a. b.* axillary buds which have continued the growth of the beet since the destruction of the original terminal bud. $\times 25$.

frequently disintegration of the parenchyma immediately inside the xylem has occurred, and in some places the phloem shows signs of the disease, either by hypertrophy of the cells, or by the blocking of most of the sieve-tubes. The fifth ring in Fig. 2 is represented by disintegrated or abnormal cells in groups. Evidently the cambium had not long been initiated here when the consequences of boron deficiency became effective. It appears that before disintegration set in, differentiation was accelerated, and vacuolization, swelling, the development of intercellular spaces, and the thickening of the cell walls took place. Some of the surrounding parenchyma cells became hypertrophied and cross divided by extremely thin walls, while other cells became crushed (Fig. 4). Immediately outside these patches of disintegrated cells is a ring of healthy, normally developed vascular tissue (Figs. 2 and 3), succeeded by two rings of cambium. The cells that have been cut off from the latter have not yet become differentiated. Evidently these last three rings are connected with the new leaves developed from the top of the beet after the restoration of boron to the nutritive solution.

Set E.

(Grown with boron until three weeks before fixation).

These plants spent the last three weeks in boron-free solutions. As in Set D, the inner secondary vascular zones show very little heart-rot, while



FIG. 2. Diagram of the vascular arcs in a sector of a transverse section taken some distance below the apex, from a beet of the D set (grown in the presence of boron except for three weeks in June and July). Healthy vascular zones shown by close parallel lines, with the vessels in outline. Diseased vascular tissue and parenchyma dotted. *a* Youngest zone of secondary vascular tissue-cells as yet undifferentiated. *b* Zone of diseased vascular tissue that must have been in process of differentiating when the disease set in. *c* Oldest vascular ring—still quite healthy. $\times 45$.

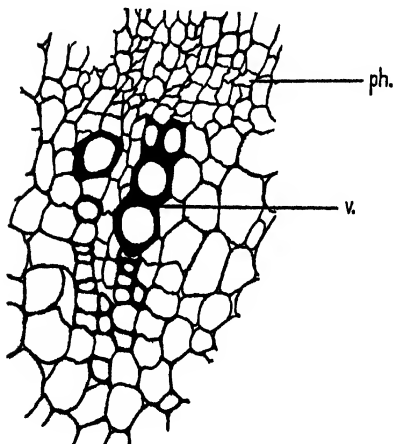


FIG. 3. A vascular strand from the arc immediately outside the diseased vascular ring shown in Fig. 2. *ph.* Phloem. *v.* Vessels. $\times 450$.

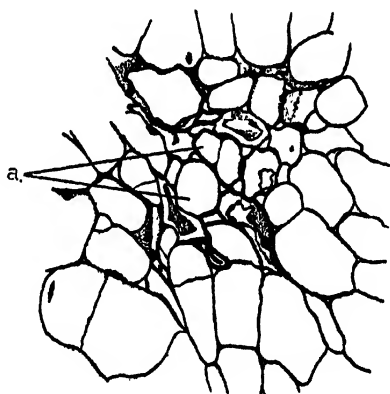


FIG. 4. Strand from the diseased vascular zone in Fig. 2. *a*. Cells which in normal conditions would have become vessels. $\times 450$.

the parenchyma adjacent to the younger vascular arcs often exhibits stages in disintegration. The outermost arc of meristematic cells, representing the activity of a new cambium, consists of an incomplete zone, several cells in width. This is diseased, the cells being crushed between hypertrophied



FIG. 5. Diagram of the vascular arcs in a sector of a transverse section taken some distance below the apex, from a beet of the E set (grown in the presence of boron until three weeks before fixation). Healthy vascular zones shown by close parallel lines, with the vessels represented in outline. Diseased vascular tissue and parenchyma dotted. *a.* Outermost arc of cambium—diseased. *b.* Innermost vascular ring—healthy. The intermediate rings are all slightly diseased. $\times 45$.

cortical cells (Figs. 5 and 6). In longitudinal section (Fig. 7), the youngest leaf-trace is seen immediately below the leaf bases, on its outward course from the growing point of the beet to the periphery.

Set F.

(Grown with boron throughout, increasing in quantity with the age of the plant).

At the close of the experiment, the axes of these plants had attained to a maximum diameter of one inch, growth of the secondary vascular rings being normal throughout the axis.

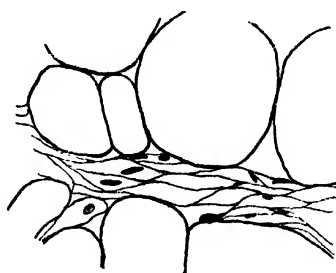


FIG. 6. Part of the crushed cambium of the outermost leaf-trace from Fig. 5. $\times 990$.

II. Anatomical details.

The meristematic tissues of the shoot are the first to degenerate as a result of boron deficiency. The apical growing point of the stem, the embryonic leaves, and the newly developed cambia rapidly disintegrate. The cells of the vascular system that were in process of differentiating when

boron was withheld appear also to be very susceptible to the omission. Frequently the cells become thick-walled but apparently not rigid, since hypertrophy of the surrounding cortical cells causes them to assume very irregular shapes (Fig. 4). Many of the cells of the ground tissue and a few



FIG. 7. Nearly median longitudinal section of a beet from set E (grown with boron until three weeks before fixation). *d.t.* entirely disintegrated tissue, including the bases of the youngest leaves and the tissue round the actual apex, which are shaded in black. *d.v.s.* diseased vascular strands, marked in thick lines. *h.v.s.* healthy vascular strands of the outer leaves and of the beet, shaded in thin lines when cut longitudinally and in dots representing the vessels when cut transversely. $\times 25$.

of those in the diseased vascular strands appear to have undergone division, and the two daughter cells are separated by an extremely thin wall. In the later stages of the disease, where older vascular arcs are affected, the cambial cells have become irregularly swollen and divided, and much of the phloem and parenchyma is completely disintegrated (Fig. 8).

Not infrequently the sieve-tubes of healthy plants contain plugs of densely staining material which presumably is callose. This is sometimes developed as a solid rod throughout the cell (Fig. 9), or disposed as a peripheral lining. These phenomena occur much more frequently in the phloem of diseased plants, where they are very typical. It may be that their development is the characteristic precursor of the subsequent degeneration in the mature tissues of vascular strands, since they are sometimes frequent in strands which do not exhibit any other signs of disease.

Root-tips of diseased plants were examined, and these showed features similar to those observed by Sommer and Sorokin (9) in boron-starved peas, *viz.* differentiated vascular tissue occurred much closer to the apex than in healthy roots, and none of the nuclei were dividing. It would appear either that there is some difference between the growing points of root and shoot, since the root-tip does not degenerate at the same time as the shoot apex,

or that the minimum amount of boron necessary for the mere maintenance of the root-tip is retained within its cells.

The cambial arcs arise centrifugally in the beet, and the peripheral ones, being the youngest, are therefore connected with the innermost

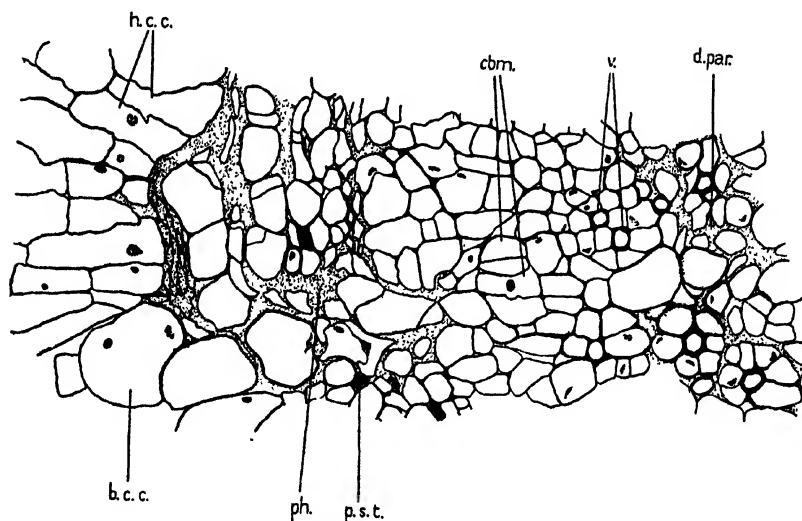


FIG. 8. Transverse section of a vascular strand from near the apex of a plant that had been deprived of boron for three weeks. *ph.* disintegrated phloem. *d.par.* disintegrated parenchyma. *p.s.t.* plugged sieve-tube. *b.c.c.* binucleate cortical cell. *v.* vessels. *h.c.c.* hypertrophied cortical cells. *cbm.* irregular and hypertrophied cells of the cambium. $\times 450$.

youngest leaves, while the older ones near the centre of the beet belong to the outer leaves. Since the leaf-bearing part of the axis is not elongated, the leaf-traces occurring on the same or approximately the same orthostichies must cross each other at some point which lies in a zone surrounding the apex, a short distance below the top of the beet (Fig. 10). The vascular anatomy in this zone is very confused. An additional complication is afforded by the fact that frequently, when the leaves are older, and have become pushed out to the periphery of the crown of the beet, a branch grows out from the original leaf-trace and forms an arc of vascular tissue outside the youngest trace on the same orthostichies (Fig. 1). Probably this accounts for certain irregularities in the vascular rings farther down from the apex.

In spite of these factors, however, the physiological individuality of the leaf-trace throughout the swollen organ is maintained, and this is reflected in the distribution of disintegrated tissue in the boron-starved plant. The youngest leaves first become blackened (Fig. 11), and hence the outermost ring of vascular tissue belonging to them first shows signs of disintegration. Although the traces pass very close to one another, they

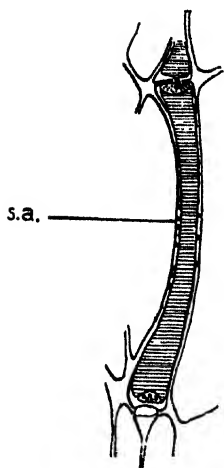


FIG. 9.

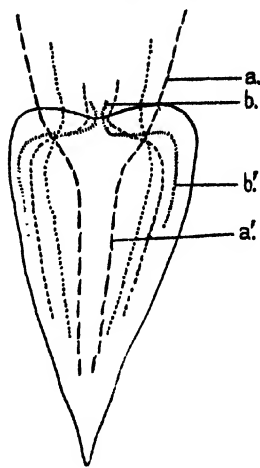


FIG. 10.

FIG. 9. A sieve-tube from a diseased plant, in longitudinal section, showing a mass of deeply staining substance filling the whole lumen. *s.a.* pits of sieve-areas on the sieve-walls. $\times 990$.

FIG. 10. Diagram to show the general course of the leaf-traces in the sugar-beet. Neither the primary vascular strand of the seedling nor the accessory strands branching off from the older leaf-traces are shown. *a*, Oldest leaf. *a'*, Oldest trace. *b*, Youngest leaf. *b'*, Youngest trace.

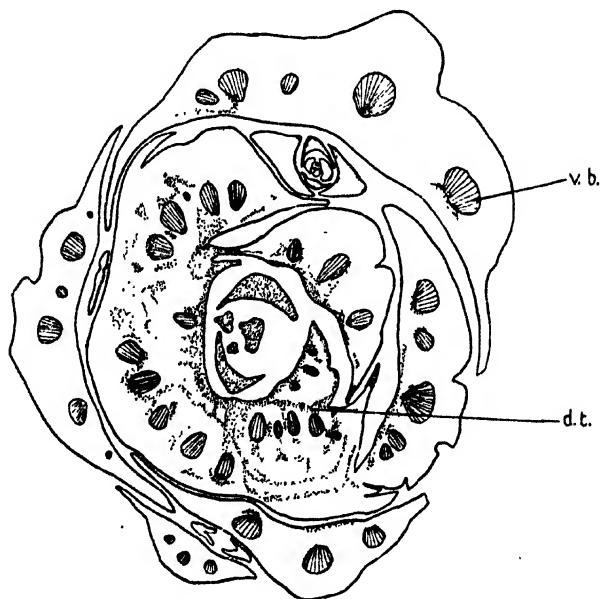


FIG. 11. Diagram of transverse section from just above the shoot apex, showing disintegration in the youngest leaves and the centrifugal spread of the disease in a plant that had been deprived of boron for three weeks. The diseased tissue is dotted. Vascular bundles are indicated in outline with line shading. *d.t.*, disintegrated tissue. *v.b.*, vascular bundle. $\times 16$.

do not 'contaminate' each other. This explains why 'heart-rot' is at first peripheral in the root-stock, and appears in the core only in the later stages (cf. Fig. 7).

The growth of axillary buds which follows after restoring boron to the nutrient solution is accompanied by the development of new cambia in the ground tissue of the hypocotyl and root. This is seen in set D (Fig. 2). There is no renewal of activity in or near those vascular zones which began to degenerate at the onset of degeneration in the leaves to which they belonged (Fig. 4). Again, the individuality of each leaf-trace of the axis is clear, for it is evident that the growth of the leaf-trace is dependent on the well-being of its own particular leaf, and no matter how much fresh growth is activated in the formation of new cambial zones, the single fact of the death of the leaf renders impossible any further development of its axial component. It is also brought to light that, in the axis, the repercussions of leaf activity include not only control of the growth of the axis, but also immediate transmission of pathological conditions, since the leaf-trace does not merely cease activity in the absence of boron, but is quickly converted into an isolated strand of diseased tissue. The undoubted connexion between the zones of secondary tissue and the leaves, together with the evidence here provided of the inability of the former to develop if the growth of the latter is checked, suggests that the axial part of the plant might be regarded as a number of fasciated leaf-traces. This can be demonstrated only in plants such as *Beta* where individual strips of cambium are associated with each leaf, and is impossible in most dicotyledons where there is only one cambium which is stimulated afresh into activity with the development of each leaf.

SUMMARY.

1. An experiment is described which confirms the results of earlier investigators, showing that boron is an essential element in the growth of sugar-beet. A concentration of 1 : 1,000,000 H_3BO_3 in the culture solution is enough for active growth and normal development.

2. It is shown that recovery in boron-starved plants involves the activation of axillary buds at the top of the beet, each of which develops its own system of secondary vascular rings independently of the others.

3. Attention is drawn to the fact that the secondary vascular zones in the beet are downward continuations of the vascular supply of the leaves, and that the influence of any factor adversely affecting the leaves is very quickly reflected in the corresponding vascular rings of the beet.

4. The apical meristem of the shoot, together with the youngest leaves and the newly developed cambia of the beet are tissues and organs which are most sensitive to boron-deficiency, and these are the first to degenerate. Cells of the vascular rings in process of differentiating, and

sporadic groups of parenchyma cells adjacent to conducting elements are also sensitive to the deficiency. Hypertrophy of the cambial cells, and also of the adjacent parenchyma cells, together with complete disintegration of the phloem, characterizes the later stages of heart-rot disease. It is suggested that plugging of the sieve-tubes is the first indication that the tissue is suffering from boron-deficiency.

5. Since the root-tip does not degenerate, but merely ceases to grow in the absence of boron from the nutrient solution, either the requirements of this meristem, or the conditions obtaining in it must be different from those of the shoot-apex.

In conclusion, the author wishes to thank Dr. W. E. Brenchley for her very helpful advice and criticism throughout the experiment.

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OBSERVATIONS ON THE EFFECT OF MOLYBDENUM ON PLANTS WITH SPECIAL REFERENCE TO THE SOLANACEAE

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(With Plate XXX and 8 Text-figures)

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I. INTRODUCTION

IN a paper(5) dealing with the nature of intracellular inclusions in plant virus diseases, Sheffield demonstrated that cytological abnormalities similar to those due to aucuba mosaic or Hy. III disease could be produced in healthy solanaceous plants by the addition of salts of molybdic acid to the soil in which they were grown. She demonstrated, further, that in the case of *Solanum nodiflorum*, treatment with molybdate resulted in an alteration of the habit of the plant, growth becoming trailing instead of erect.

The present investigation was undertaken with a view to extending our knowledge of the effect of molybdenum salts on plant growth from the nutritional and physiological aspects. Results of the type looked for were not forthcoming, but several points arose which justify description. Plants, chiefly members of the Solanaceae, were grown in pot or water

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culture during the three summers 1934-6, molybdenum treatment being carried out with a wide range of concentrations, as little was known of the effect of the element. The sodium salt was used throughout. Observations were made at frequent intervals during growth, and material from the cultures was examined microscopically and chemically.

II. CHEMICAL METHODS

The chief reactions required in this work were tests for the presence of molybdenum and tannin.

A. *Molybdenum*

The most satisfactory test for molybdenum is the production of a magenta pink colour on the addition of xanthate solution. The latter is made by adding excess carbon disulphide to a concentrated solution of caustic potash in absolute alcohol, followed by sufficient 30 per cent acetic acid to cause a yellow precipitate. Freshly made solution is used as it deteriorates rapidly. When testing plant material it was necessary to add a few drops of hydrochloric acid (2 or 10 per cent equally good) until the liquid turned milky, otherwise the characteristic pink colour failed to appear although molybdenum was present. The need for this addition of hydrochloric acid was unexpected, as the test worked perfectly *in vitro* with a molybdate solution, but, as will be seen later, much of the molybdenum within these plants was combined with tannin, and test-tube trials with such a combination also gave negative results unless hydrochloric acid was added. Similarly, ashed material from plants receiving heavy molybdenum dressings gave a blue and not a pink colour when the xanthate solution was applied, failing the addition of hydrochloric acid.

B. *Tannin*

Tests for tannin were made with two reagents: (i) 10 per cent ferric chloride solution containing sodium acetate, and (ii) 1 per cent osmic acid. With the first, a green-black, and with the second a black coloration was obtained. Both tests were usually employed.

III. EXPERIMENTAL PLANTS

A. *Potato*

(a) *Pot experiments.*

Three series of pot experiments were carried out with potatoes, a mixture of Rothamsted and "old cucumber" soil with an addition of approximately 10 per cent sand being used, 22 kg. in all per pot. Additional

manures were applied in the first series but, as the plants grew so luxuriantly, they were omitted in the later experiments for fear of masking the effect of the molybdenum treatment. The latter was given as soon as all plants were showing above the soil. In external appearance, little difference could be seen between the shoots of plants grown with or without molybdenum, although those receiving the heaviest dressings were slightly later in developing than the controls. The tubers were chiefly affected, treatment with over 2 g. of sodium molybdate per pot causing a marked change in colour, the skin turning a golden to reddish brown. A form of corky scabbing, especially pronounced where the larger dressings had been applied, often accompanied the discoloration.

(b) *Microchemical investigations.*

The two varieties grown, Epicure and Eclipse, are normally white skinned, and the yellowing of the tubers was clearly correlated with the molybdate treatment. The discoloration was confined to the epidermal and subepidermal regions of the tuber, and was traced to the presence of bright yellow globules which occurred in large numbers in these tissues (Text-fig. 1 A).¹ The globules were not found in any part of tubers from control plants. Their form was generally globular, closely resembling oil drops, but a variety of types occurred. A lighter rim frequently bounded the central and main part of the globule, which might be finely granular (Pl. XXX, fig. 2) or consist of a group of small globules (Text-fig. 1 B). Usually one such globule occurred in a cell, but several distinct individuals or groups of small globules together were sometimes to be seen, the variety in size being very considerable. Yellow masses of a granular type were also found which gave similar chemical reactions to those of the globular form (Text-fig. 1 C). A number of chemical tests were carried out chiefly on fresh tubers, but confirmatory results were obtained with fixed and imbedded material.

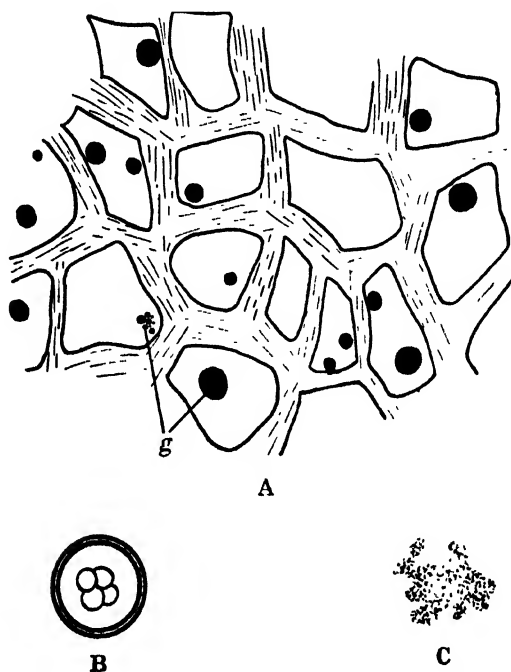
Reactions for fatty or protein compounds were negative. Acids caused decolorization, that induced by hydrochloric acid being more rapid than that with either acetic or oxalic acids. Alkalies such as caustic potash and ammonia also decolorized the globules. On the addition of xanthate solution, the yellow bodies immediately turned a bright magenta pink colour, showing the presence of molybdenum. Further, the globules turned olive green with ferric chloride solution and black with osmic acid, indicating the presence of tannin. A yellow or reddish yellow compound of molybdenum and tannin, which decolorizes with

¹ All text-figures were drawn with camera lucida.

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oxalic acid, is known to occur(2), and since, as will be seen later, the yellow globules were situated in those tissues of the potato tuber which normally contain abundant tannin(1, 3), it seemed probable that the bodies were composed of this tannin-molybdenum compound.

The various parts of the plants were examined at intervals during growth to determine the stage at which the yellow globules were produced, the molybdenum dressing necessary for their formation, and



Text-fig. 1. A, epidermal stripping showing yellow globules (g) from tuber of potato grown with sodium molybdate. ($\times 66$.) B, globule with smaller globules at centre. ($\times 500$.) C, granular form of yellow body. ($\times 500$.)

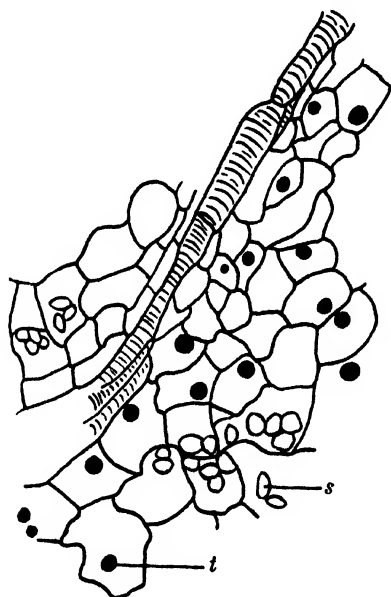
their distribution in the plant tissue. Thirteen days after an application of a 5 g. dressing of sodium molybdate had been given per pot of 22 kg. soil, globules were evident in the underground but not in the aerial parts of the stem, although small isolated granules of a molybdenum nature were occasionally to be found in all parts of the shoot. No yellow bodies or molybdenum granules were seen in the control plants, nor at this stage in those receiving the smaller dressing of sodium molybdate (2.5 g. per pot). Twenty-three days later, the molybdate dressings were repeated, the controls still receiving none. By the time tubers had begun

to form, yellow globules were abundant in the epidermal layers of the underground parts of the stem of all treated plants. None, however, were to be seen in the aerial parts nor in the control plants. The globules were confined to the epidermal layers and were particularly plentiful where buds were emerging. As soon as the haulms had died down the tubers were lifted and stored in a cupboard in the laboratory until they sprouted. Yellow globules were still as plentiful as when the crop was first lifted, but there was no change in their distribution, nor had they formed in the control plants. Globules were specially plentiful at those points in the epidermis where new growth was taking place (Pl. XXX, figs. 1 and 2). In the emerging bud they occurred in large numbers, being most prevalent in the central tissue between the vascular strands and in the strands themselves, a few only being found in the epidermal layers. They were restricted to the lower region of the new shoots, i.e. at or just above the point of emergence from the tuber, and although molybdenum was detectable in the vascular tissue of the upper parts and also in the conducting tissue of the old tuber, it was in the form of solution and not as definite globules.

In view of the probable tannin-molybdenum constitution of the yellow bodies, the normal distribution of tannin in the tubers of control plants at the sprouting stage was determined. Abundant tannin was detected in the undifferentiated meristematic tissue and at the apices of young protecting leaves, these tissues turning a brownish black or black colour with osmic acid, and a deep green-black colour with ferric chloride. Occasionally with ferric chloride and invariably with osmic acid, dark globules appeared in the central and vascular regions, and to a less extent in the epidermis of the lower parts of the bud, i.e. in precisely the same tissues as where the yellow globules had been found in the molybdenum-treated plants (Text-fig. 2). The size and appearance of the two types of body were also similar, both showing a dark central region surrounded by a lighter layer. These results were obtained before the writer's attention had been drawn to Hartmann's work (3), and confirm his findings, both as regards the form and distribution of the tannin in the tuber. It is interesting that he records both a granular and globular form of tannin, as the fact that two distinct types of yellow body were to be found, which reacted similarly to xanthate and ferric chloride, had offered some difficulty. Since the distribution of tannin in the normal tuber was identical with that of the yellow globules in plants treated with molybdate, it seemed justifiable to conclude that the molybdenum taken in by the plant had combined with the tannin already present and that

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the yellow globules were the result of a direct union of the molybdenum and tannin *in situ*. In the old parent tubers molybdenum had been noted in the vascular tissue, and as this would be conducting food materials to the actively growing buds where the tannin is concentrated, a combination of the two substances might occur. Apparent proof of this was obtained by producing artificially similar yellow globules in plants which had not been treated with molybdenum during growth. Fresh seed potatoes of the same varieties as had been used in the experi-



Text-fig. 2. Section through conducting tissue just below developing bud in tuber of control showing potato tannin globules (*t*) after treatment with osmic acid. *s*=starch grains. ($\times 375$.)

mental pots were sprouted and longitudinal sections cut through emerging buds. After examination to ensure that no yellow globules were present, a solution of sodium or ammonium molybdate was run in on to the slide. Small dark round bodies, some of which turned bright yellow, appeared in the central tissue of the young bud and in the epidermis. Although they did not occur so plentifully their appearance and distribution seemed identical with the tannin bodies in the control tubers and with the yellow globules in the tubers of molybdenum-treated plants. In very young buds globules did not appear, but the meristematic tissue at the apex turned a golden yellow colour which

disappeared on the addition of oxalic acid. Treatment with an alkali, such as caustic potash, in place of the molybdate solution resulted in a greenish yellow colouring of the buds, so that the reaction with the sodium molybdate was not merely due to its alkalinity. Tubers freshly dug from the field in autumn showed none of these yellow globules whether they were from clean or virus-affected plants, nor was there any sign of such bodies in unsprouted seed potatoes purchased in the spring.

On sprouting, the potatoes developed a certain amount of anthocyanin pigment, and tubers from plants treated with molybdenum occasionally showed the presence of large blue granules in the subepidermis, i.e. the tissue where the pigment was most plentiful. The nature of these blue granules will be discussed under the section dealing with the tomato, where such bodies were frequent.

The potato tuber was the simplest material for chemical tests and yielded some of the most clearly defined results owing to the comparative scarcity of complicating pigments such as chlorophyll or anthocyanin. As the tubers could be stored without deterioration, reference could be made to the same batch of material over a wide range of time, thus affording a useful means of checking chemical tests on other types of plant material.

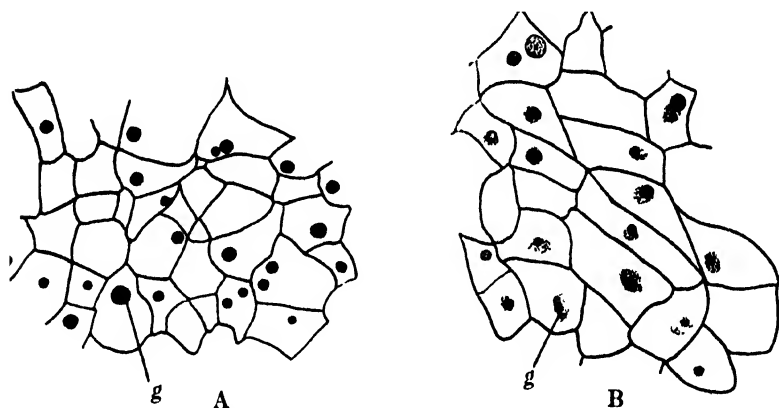
B. *Tomato*

(a) *Pot- and water-culture experiments.*

Since little was known concerning the effect of different quantities of molybdenum on the tomato plant, the preliminary experiments were carried out with a fairly wide range of concentrations. In the pot-culture work, dressings of 4, 2, and 1 g. respectively of sodium molybdate, were applied in solution per pot of 18.18 kg. soil, 12 days after the young plants, one per pot, had been set up. Except for the three controls each treatment had six replicates. No basal manures were added as the soil was "old cucumber" known to be rich in nitrogen and other nutrients. Individual variation was considerable, but the 4 g. dressing was always toxic, and several of the plants soon died, their leaves turning a purplish grey colour with a tendency to curl over as if flaccid. The plants that survived showed somewhat different symptoms, the whole of the shoot becoming a golden yellow. The effect was most marked at the apex, where the youngest leaves turned a bronze shade, the lamina being so reduced that it consisted almost entirely of midrib. The weak, straggly habit and the drooping of the leaves persisted throughout the life of the plant (Pl. XXX, fig. 3), the older leaves developing a mottled appearance

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remarkably like that induced by a virus disease. As Sheffield (5) had shown that inclusion bodies were formed in plants treated with molybdenum similar to those affected with virus disease, tests were made to see if the mottle-leaved molybdenum-treated plants were capable of transmitting virus. Dr Sheffield kindly carried out inoculations with the juice of the leaves of the most severely poisoned tomatoes in these pots. Six healthy (i.e. virus-free) tomato and ten leaves of two *Nicotiana glutinosa* plants were inoculated, but no symptoms of virus or any other affection developed. It seemed unlikely, therefore, that the mosaic-like appearance of the leaves of the molybdenum-treated plants was due to any transferable agent. The 2 g. dressing induced similar but less pro-



Text-fig. 3. A, epidermal stripping from back of leaf of tomato grown with sodium molybdate showing yellow globules (*g*). ($\times 75$.) B, similar stripping showing more granular appearance of globule (*g*) when stained pink after treatment with xanthate solution. ($\times 75$.)

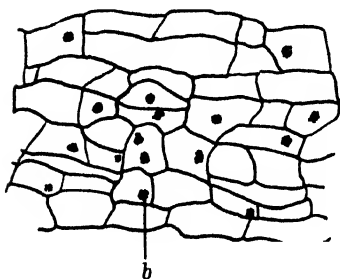
nounced symptoms, while plants receiving only 1 g. per pot were practically indistinguishable from the controls, all of which grew into large healthy plants. Fruit formation and maturation occurred throughout, even on quite badly poisoned plants, though in these it was somewhat retarded.

In the water-culture experiments a series of concentrations was tried ranging from 1 : 12,500 to 1 : 250,000 of the element Mo in the nutrient solution. The strongest dose induced symptoms very similar to those described for the pot cultures, the bright yellow or bronzed colour of the leaf, its reduction in size and the general flaccid and straggling appearance of the plants being the chief features. A reduction in root development and a discoloration of a yellowish brown nature invariably accom-

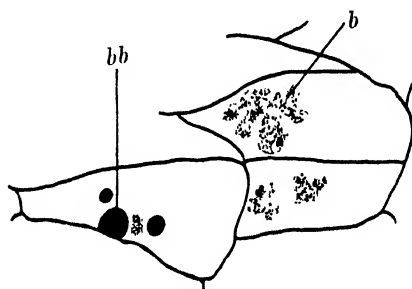
panied an injured shoot. As in the pots, fruiting took place in all plants, though it was somewhat retarded and reduced in the sets receiving high concentrations of molybdenum.

(b) *Microchemical investigations.*

Numerous bright yellow globules were found in the epidermal layers, chiefly on the lower surface, of the bronzed leaves of molybdenum-treated plants (Text-fig. 3 A). They reacted similarly to the chemical tests as the globules in the skin of the potato tuber, so that it seemed highly probable that the two sets of yellow bodies were identical in composition, viz. of a tannin-molybdenum nature (Text-fig. 3 B). None of these yellow globules were to be seen in the leaves of young tomato



Text-fig. 4.



Text-fig. 5.

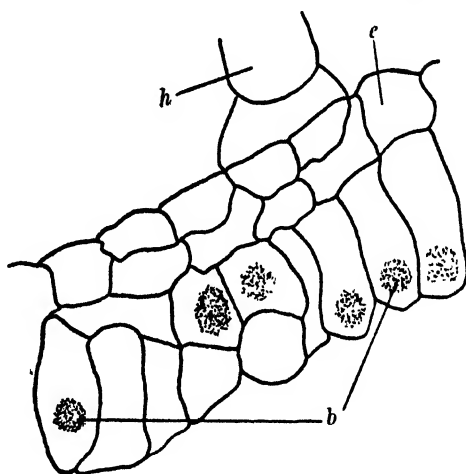
Text-fig. 4. Epidermal stripping from hypocotyl of tomato grown with sodium molybdate showing blue granules (*b*). ($\times 75$.)

Text-fig. 5. Individual cells of epidermal stripping from hypocotyl of tomato grown with sodium molybdate showing granular (*b*) and globular (*bb*) form of the blue body. ($\times 375$.)

seedlings, or of more mature plants not showing severe symptoms of molybdenum poisoning. Their shoots, however, contained numerous blue accumulations (Text-figs. 4 and 6) which were most plentiful in plants grown with the larger quantities of the element, and were proved not to be connected with any alkaline condition of the sap due to the molybdate treatment. Very rarely blue granules occurred in control plants, but it is considered that some trace of Mo as impurity must have been responsible for this. Most of the blue bodies were roughly spherical in shape, of a loose granular appearance and not generally bounded by a clearly defined outline like the yellow globules (Text-fig. 5). There was usually one per cell, and though the colour was generally blue, some variation in this respect occurred. When the granules occurred in cells containing anthocyanin pigment, they were usually purple, but in other

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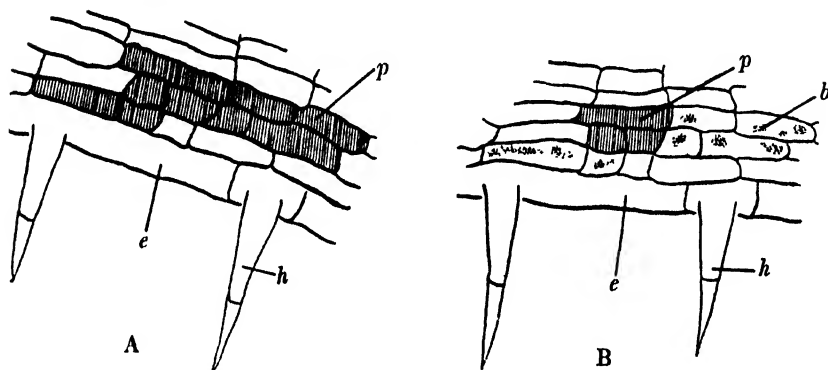
cases they were blue, greenish yellow or yellow, though there was never any approach to the golden yellow colour of the other type of globule. In the leaves the blue granules were most plentiful near the apices, at the margins and in the veins, their distribution thus differing from that of the yellow globules which were practically confined to the epidermal layers. Further, they appeared at an earlier stage in the life of the plant than the yellow bodies, being noticeable even in the cotyledons. In the petiole the blue granules occurred chiefly in the subepidermal layers (Text-fig. 6), while the yellow were to be found in the cortex. Very occasionally the two types of body occurred in the same cell.



Text-fig. 6. Transverse section of petiole of tomato grown with sodium molybdate, showing blue granules (*b*) in subepidermis. *e* = epidermis, *h* = hair. (× 375.)

Tests were made to determine the chemical nature of these blue granular masses. Hydrochloric or acetic acid caused them to disappear with the formation of a pink solution, whereas with alkalis, such as caustic potash or ammonia, they collapsed and faded, sometimes with a momentary formation of a blue solution. Alcohol also caused decolorization, but the action took place more slowly than with alkalis. No indication of the presence of tannin was obtained on the addition of ferric chloride, though a pink colour developed gradually, doubtless due to the acid nature of the ferric chloride. Results from the xanthate test for molybdenum were negative, with occasional suggestion of a positive reaction which was too indefinite to credit. This negative result was difficult to accept owing to the association between the formation of blue bodies and the application of sodium molybdate to the plants in which

they were found. Two explanations suggested themselves, viz. either the molybdenum, if present in the granules, was too closely combined to be able to react to the xanthate test, or the presence of molybdenum induced their formation in an indirect manner. Unsuccessful attempts were made with acid and alkali to break up the bodies before applying the xanthate test. The tomato leaf is rich in anthocyanin pigment which is naturally purple, but turns pink under acid, and blue, green or yellow under alkaline conditions. Since the xanthate test (which, when positive for molybdenum, induced the formation of a bright magenta-pink colour) worked satisfactorily only under acid conditions, which on their own account turned the pigment pink, erroneous conclusions were easily drawn. Caution in interpreting results was therefore required unless the



Text-fig. 7. A, epidermal stripping from back of leaf of tomato control plant. *e* = epidermis, *h* = hair, *p* = purple pigment. ($\times 75$.) B, same tissue after treatment with sodium molybdate. *b* = blue precipitate, *e* = epidermis, *h* = hair, *p* = purple pigment. ($\times 75$.)

two shades of pink were available for simultaneous comparison. The addition of xanthate solution to yellow globules in the presence of acid always resulted in a retention of their structure even with the change of colour, whereas in the case of the blue bodies collapse occurred.

An attempt was made to approach the problem from the synthetic side. Sections from leaves of control plants, containing abundant anthocyanin pigment but no blue bodies, were treated with sodium molybdate solution, similar sections being treated with alkali for comparison. In the latter case the usual colour change to blue, green or yellow occurred, the coloured sap collecting into large circular masses as plasmolysis set in. With the molybdate solution, on the other hand, although several cells behaved as if an alkali had been added, in others a marked blue granular precipitate developed (Text-figs. 7 A and B). This was repeated

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a number of times, so that it was tempting to deduce that the granular blue bodies found in the molybdenum-treated plants arose as a result of a precipitation between the molybdenum salt and the anthocyanin pigment in the sap. The circular form of the naturally occurring accumulation would readily be produced by the normal protoplasmic movement within the cell. Further confirmation of this point will be found on p. 488. The earlier failure to get a positive result with the xanthate reaction was probably due to the fact that the molybdenum was in combination with a substance that turned a very similar colour with the reagent so that the molybdenum reaction was masked.

C. Solanum nodiflorum

(a) Pot- and water-culture experiments.

Solanum nodiflorum grew well in soil culture in pots, but was at first difficult to establish in water culture, as its requirements regarding nutritive solution were unknown. It was clear from the start that this species was peculiarly sensitive to the influence of molybdenum. Two series of pot culture were carried out. In the first, single plants were set up in pots containing 2.5 kg. of a mixture of "old cucumber" soil and 10 per cent sand, sodium molybdate being added at the rate of 0.5-1.5 g. per pot, there being six replicates for each treatment and three control pots. All dressings of 1 g. or more rapidly proved toxic or fatal, the leaves of the plants turning yellow or flaccid. The 0.5 g. dressing, on the other hand, had little or no effect. In the second series, larger pots were employed, each being filled with 8.6 kg. of a mixture of heavy Rothamsted and "old cucumber" soil. Dressings of sodium molybdate ranging from 0.25 to 1.0 g. were applied 8 days after the plants, one per pot, had been set up. Each treatment had four replicates, and there were four controls. After the lapse of 40 more days, those which had received the 0.75 or 1.0 g. dressing were given a further 0.25 and 0.5 g. sodium molybdate respectively. As in the first experiment 1 g. of sodium molybdate per pot definitely checked growth at the start, but in this case serious or fatal effects from the dressing did not occur. Some yellowing was observed in the leaf, but, on the whole, almost complete recovery from the initial check took place. The difference in degree of toxicity of the same dressing of molybdate in these two experiments is no doubt due to the alterations made in type and quantity of the soil used, and serves to emphasize the danger of specifying any particular dressing as toxic or lethal without sufficient qualification regarding the other experimental conditions.

In the water-culture work a wide range of concentrations of molybdenum was tried. 1 : 25,000 Mo was toxic, in some cases proving fatal, but there was some indication that the degree of injury was correlated with the age of the seedling, the younger ones being the more susceptible. Some toxicity was also evident with concentrations of 1 : 100,000 Mo, but here, as in the pot culture, the degree of injury produced varied considerably with the other experimental conditions. The principal symptom was a marked yellowing of the shoot accompanied by a flaccid appearance of the leaves, and a general reduction in the size of the plant.

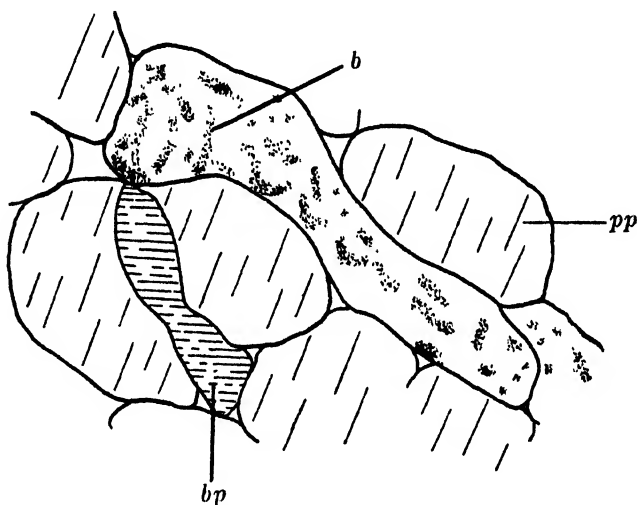
In none of these experiments did the molybdenum-treated plants develop a trailing habit as described by Sheffield (5), and recently confirmed by her. Sheffield's plants were grown in a heated glasshouse with high humidity, and were of a soft, succulent and rapid-growing type, whereas those now under discussion were grown in a different soil and in an unheated glasshouse with a very dry atmosphere and were slow growing, stiff and woody. Thus the conditions under which the two sets of investigations were carried out were entirely different, and this may account for the discrepancy between the results. Comparative investigations are in progress which may elucidate the matter.

(b) *Microchemical investigations.*

Internal changes occurred in almost normal-looking plants. Yellow globules, similar to those found in potato and tomato tissues, but not nearly as plentiful, occurred in the leaves of *Solanum nodiflorum*, and they could be produced artificially in sections of the leaf tips of either control or molybdenum-treated plants by the addition of sodium molybdate solution. Blue granular accumulations, similar to those in the tomato, occurred in large numbers in the cells containing anthocyanin pigment of plants treated with molybdate, and very occasionally also in control plants. The pigment occurred chiefly in the subepidermal layers, i.e. in the palisade of the leaf and the outermost layer of the cortex in the stem. In the peduncles bearing ripe berries the pigment was much more plentiful, being found throughout the cortex and pith as well. In the controls the pigment was invariably purple, but in plants treated with sodium molybdate it was often blue, both forms of pigment turning pink on the addition of acid. When treated with xanthate no positive evidence was obtained that the granules contained molybdenum, although the characteristic pink colour developed immediately in the conducting tissue and in certain isolated cell contents such as those in the leaf hairs. The formation of a blue granular precipitate was induced in the

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pigmented portion of leaves from control plants by the addition of sodium molybdate solution (Text-fig. 8), just as in the tomato, and it was possible to test the matter even further in the case of *S. nodiflorum* owing to the more plentiful development of pigment of this species. An extract of cell sap rich in anthocyanin was made by crushing berries of *S. nodiflorum* in a glass mortar in the cold with a little distilled water and filtering. With hydrochloric acid the extract turned bright pink, and with caustic potash a succession of colours were obtained in the order blue, green and yellow. The addition of sodium molybdate solution brought about a precipitation of a purplish blue colour, the formation of



Text-fig. 8. Epidermal stripping from back of leaf of *Solanum nodiflorum* control plant after treatment with sodium molybdate. *pp* = purple pigment, *bp* = blue pigment, *b* = blue precipitate. (All cells contained purple pigment before treatment.) ($\times 375$.)

which took place more readily if some sodium chloride were added before the molybdate. These reactions *in vitro* were similar to those in the fresh leaf tissue of both this plant and the tomato, and it seems probable that the origin of the blue granules depended on a precipitation of the anthocyanin pigment by the molybdenum salt.

The artificially produced precipitate when mounted on a slide dissolved in excess water, disappeared with formation of a pink solution on the addition of hydrochloric acid, and dissolved rapidly with temporary formation of a blue solution on the addition of caustic potash. Similar reactions were obtained with the artificially produced granules within the cell itself, so that their nature seemed to be identical. Some

explanation for the range of colour among the naturally occurring granules is provided by these tests *in vitro*. Purple granules are found in the presence of excess purple pigment where precipitation is presumably incomplete, a true blue colour being obtained under more definitely alkaline conditions as would set in after longer treatment with sodium molybdate. The green and yellow variants might be regarded as the result of prolonged alkaline reaction, just as the normal purple pigment turned first blue but ultimately green or yellow on the addition of caustic potash or ammonia. Confirmation of this was provided by the addition of further sodium molybdate to tissues from molybdenum-treated plants, a procedure which caused the purple granules already present to turn a greenish blue colour. A phenomenon was observed during these microchemical investigations, which emphasizes the need for caution in technique. Strippings taken from the skin of ripe *S. nodiflorum* berries where the pigment is normally a rich purple contained some cells which were blue. Their general appearance was not characteristic of molybdenum-treated tissue, there were no definite granular accumulations, and they generally occurred at the edge of the sections only. The change in colour was not brought about by exposure to air, nor by the water used as mountant, but was eventually traced to the use of metal forceps and scalpel. If these implements were allowed to dip into a drop of juice expressed from a ripe berry on to a glass slide, the juice rapidly turned blue, whereas a similar drop left untouched remained purple indefinitely. The constituent of the metal responsible for this change was not determined, but it was apparently not manganese or chromium. Molybdic acid turned the juice royal blue, but the characteristic prussian blue colour of the granules was only obtained after the addition of alkali.

D. Barley

(a) *Pot- and water-culture experiments.*

Two years' experiments were carried out with barley in pot and water culture. This crop showed much less response to molybdenum than the solanaceous species, but certain effects were obtained that were clearly correlated with the treatment. In pot culture, dressings of sodium molybdate were applied in a succession of waterings as soon as the plants had become well established (approximately 1 month from sowing), the total amounts supplied ranging from 0.5 to 8 g. per pot of 10 kg. Rothamsted soil. Little or no difference in rate of growth, time of maturing and final dry weight was shown between the various treatments, but all the

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plants receiving 4 g. or more of sodium molybdate evinced a marked tendency to fall over from the base when full grown; in fact all these plants required staking towards the end of the experiment, a most unusual procedure for barley. Apart from this feature no toxic or beneficial effects of molybdenum were noticeable. In water culture symptoms of poisoning were obtained in both shoot and root of plants receiving 1 : 100,000 Mo or more (Pl. XXX, fig. 4), though the effect was more pronounced in the 1934 than in the 1936 season. Toxic effects were shown by a tight rolling up of the shoot in the early stages and a stunting of both the shoot and root system. With severe injury the shoots turned a greyish green colour in the young stages, and a bright golden yellow as the leaves matured in place of the usual dull straw colour.

(b) *Microchemical investigations.*

The anthocyanin pigment that normally occurs in the leaf sheath of barley is purple, but in the plants grown in water culture containing a high concentration of sodium molybdate (e.g. 1 : 10,000 Mo) the pigment was blue. The difference in colour was so definite that the molybdenum-treated plants could readily be detected by their external appearance. A blue granular precipitate was frequently present in the cells of such sheaths which possibly represented an early stage in the formation of the blue spherical granules that were so plentiful in *S. nodiflorum* and tomato. Yellow globules that superficially resembled those occurring in the potato and tomato were seen in the leaves, but, as they failed to react positively to the xanthate test, they were of a different nature. The conducting tissue of the leaves of molybdenum-treated plants invariably showed the presence of molybdenum, so that the failure of the globules to give the characteristic pink colour was convincing.

E. *Various plants*

The broad bean (*Vicia faba*) was the only other plant with which definite experiments were carried out. This species was much less sensitive to molybdenum than were the solanaceous plants, and, although definite reduction in size and discoloration of the roots took place with doses of 1 : 50,000 Mo or more, no characteristic toxic symptoms appeared. Molybdenum was detected in quantity in the conducting tissue of the leaves of the treated plants, but no blue granules or yellow globules were found.

The various weeds which appeared in the pots were allowed to continue growth, and although parallel plants in treated and untreated soils

were unfortunately not available, a few observations were made upon them. In the leaves of *Sonchus arvensis*, *Capsella bursa pastoris*, *Rumex* sp. and *Polygonum aviculare*, grown with a molybdenum dressing, yellow or blue bodies were absent. In *Ranunculus* sp., numerous blue granules were present near the leaf apices and in the veins, just as in the tomato. Although only a single specimen was available, it seems significant that *Ranunculus* was the only species which, at the time of investigation, showed the presence of anthocyanin pigment in its leaves, thus supporting the idea that the blue granules arose as a precipitation of the pigment by the molybdenum.

IV. DISCUSSION

Although knowledge concerning the importance of minor elements in plant life is rapidly increasing, it is still far from complete, and in the case of a number of elements quite rudimentary. Botanical interest in molybdenum has only recently been aroused. Apart from the fact that it occurs in plant material⁽⁶⁾, where its distribution may be of interest⁽¹⁾ and that it is toxic when presented in too large quantities, little was known about its effect on higher plants until Sheffield⁽⁵⁾ demonstrated that it induced the formation of intracellular bodies identical in appearance with the inclusion bodies that accompany virus disease.

In the present investigation, attention has been confined to determining some of the effects of molybdenum treatment on the plant, both internally and externally, in the hope of making some contribution to the wider issue, i.e. the function, if any, of the element in the metabolic processes. From the chemical point of view, molybdenum has the property of combining with an unusually large number of substances, and it is hardly surprising that compounds of molybdenum occurred in the tissues of plants treated with this element. Two such compounds, the one with tannin, the other with anthocyanin, resulted in the formation of definite bodies within the plant cell. Their numbers were so large that their presence generally altered the external appearance of those parts of the plant in which they occurred. The shoots of tomato and the tubers of potato, for example, turned a golden and reddish yellow colour respectively when the yellow globules of tannin and molybdenum were present in any quantity, while the leaves of *Solanum nodiflorum* and the leaf sheaths of barley took on a blue rather than purple appearance when the molybdenum had reacted with the anthocyanin pigment.

The formation of these compounds does not appear to be the cause of the injury which results from the stronger doses of molybdenum, as

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toxic effects occur in plants such as the broad bean where no such compounds are found; conversely, the blue granules may be present to quite a considerable extent in apparently healthy tomato plants. It is, moreover, hardly to be expected that the precipitation of part of the anthocyanin pigment should seriously affect growth. Similarly, the formation of inclusion bodies is not the cause, but a symptom, of the diseased condition of a plant attacked by virus. Molybdenum, therefore, although not yet in the ranks of those elements known to be essential for growth, evidently plays a part in the cytological as well as morphological behaviour of the plant, but its precise function remains to be determined.

V. SUMMARY

1. In view of the similarity between certain cytological changes induced by virus disease and treatment with molybdenum, pot- and water-culture experiments were carried out to determine further the effect of this element on plant growth. Sodium molybdate was used throughout.

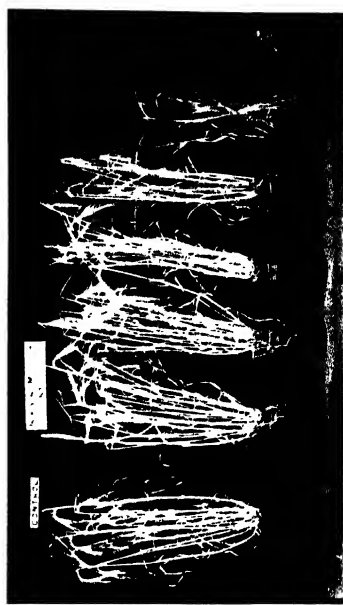
2. Toxic symptoms were produced with the larger dressings of molybdate, injury being shown at much lower concentrations in solanaceous species than in barley.

3. The shoots of tomato and *Solanum nodiflorum* turned a golden yellow, and potato tubers a reddish yellow colour when the plants were grown with the larger quantities of molybdate.

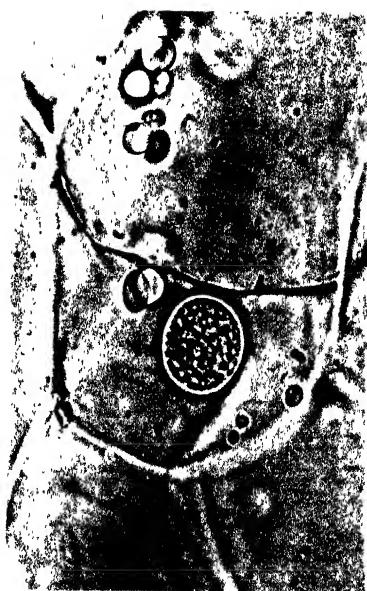
4. These colour changes were shown to be due to the presence of yellow globules of a tannin-molybdenum compound which had formed within the tissues.

5. Blue granular accumulations occurred in large numbers in molybdenum-treated plants. Their distribution was confined to tissues that contained anthocyanin pigment, and their composition was apparently of an anthocyanin-molybdenum nature.

Acknowledgements are due to Dr F. M. L. Sheffield for taking the photomicrographs and for her interest in the progress of the work, and to Mr V. Stansfield for the remaining photographs.



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EXPLANATION OF PLATE XXX

- Fig. 1. Photomicrograph of section through conducting tissue just below developing bud in tuber of potato grown with sodium molybdate. *g* = yellow globule, *s* = starch grains. ($\times 424$.)
- Fig. 2. Photomicrograph of single yellow globule showing granular centre surrounded by light rim. ($\times 844$.)
- Fig. 3. Tomatoes grown in pot culture. L-R: control, dressings of 1, 2, and 4 g. sodium molybdate per pot.
- Fig. 4. Barley grown in water culture. L-R: control, 1 : 1,000,000; 1 : 250,000; 1 : 50,000; 1 : 25,000; 1 : 10,000; 1 : 2,000 Mo.

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THE INFLUENCE OF BORON ON THE SECOND YEAR'S GROWTH OF SUGAR BEET AFFECTED WITH HEART-ROT

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(With Plates XXXI and XXXII)

AN attack by a disease, of which the symptoms were characteristic of "heart-rot", shown by death of the apical bud, occurred in a field of sugar beet on the Rothamsted farm in 1935, the attack being very localized in its occurrence. It was particularly severe in an area of about 1 acre on the south side of the field, and in the remaining area of about 3 acres of beet affected plants were only occasionally found. The severely affected area formed part of a field experiment in which the effect of sowing date, spacing of the rows and of sulphate of ammonia were being studied (for full details see *Rothamsted Annual Report*, 1935, p. 182).

Estimates of the intensity of attack on the different plots of this experiment were made on 20 November. One row of plants was selected at random from each plot, and the total number of plants (ranging from 117 to 160) and the number of affected plants were counted. An attempt was made to distinguish between severely and slightly affected plants. The slightly affected plants had a dead and blackened apical bud, but many leaves of the main axis were still healthy. In the severely affected plants there were very few leaves of the main axis remaining, and the top of the plant had a dense bushy growth of leaves developed from axillary buds. The classification into slightly and severely affected plants was somewhat arbitrary, for, actually, there was an almost continuous gradation of severity of attack.

Table I shows the number of affected plants expressed as percentage of the total number of plants. Only the main treatment effects are shown, as there was no evidence of any interactions between treatments.

Table I

Number of plants affected by heart-rot as percentage of total

Date of sowing	15 March	18 April	16 May
	19.5	13.7	4.2
Spacing of rows	10 in.	15 in.	20 in.
	10.2	12.7	14.4
Sulphate of ammonia N per acre	0 cwt.	0.3 cwt.	0.6 cwt.
	14.5	12.1	10.7

S.E. ± 2.30 .

The number of affected plants per cent on different plots ranged from 0 to 35, with a mean of 12.5. The differences between sowing dates were significant, the severity of attack decreasing steadily with later sowing. The effects of spacing of the rows and of sulphate of ammonia were not significant; but the results show a tendency for the number of affected plants to increase with wider spacing, and to decrease with increase in the amount of sulphate of ammonia applied.

Table II gives the ratio of the number of plants showing severe symptoms to the number showing slight symptoms.

Table II

Ratio. Number of plants severely affected: number slightly affected

Date of sowing	15 March	18 April	16 May
	1.24	0.79	0.53
Spacing of rows	10 in.	15 in.	20 in.
	0.76	0.84	0.96
Sulphate of ammonia N per acre	0 cwt.	0.3 cwt.	0.6 cwt.
	0.81	1.05	0.69

The effects of treatment on this ratio were closely parallel to those on the number of affected plants. The two measures of severity of attack confirm each other; where the number of affected plants was high, a higher proportion of affected plants showed severe symptoms. It is probable that badly affected plants were those in which the disease appeared at an early date, for the severe type in which all or nearly all the leaves of the main axis have disappeared is merely a later stage in development from the slight type, the leaves produced from the apical bud before its death having died in the normal process of senescence. This is in harmony with the observed fact that the earliest sowing, which had the longest growth period, had also the highest proportion of severely affected plants.

Sections cut across the top part of the crown or through affected stems showed signs of disintegration in the parenchyma and in the neighbourhood of the vascular bundles, recalling the symptoms of boron

deficiency in *Vicia faba*. With a view to obtaining definite information as to whether this attack of disease in sugar beet was heart-rot caused by boron deficiency, a selection of typical plants was lifted and transplanted into sand cultures in pots with various treatments.

Plants showing no symptoms and others badly or slightly affected were taken from plots with 15 in. spacing, sown in mid-April, some receiving no nitrogen and others receiving single and double dressings of sulphate of ammonia (0.3 and 0.6 cwt. of nitrogen per acre). The plants were placed singly in pots holding about 25 lb. of sand and either left without boron or given 0.25 or 0.5 g. boric acid. The scheme therefore embraced all combinations of

No symptoms	No nitrogen	No boron
Slightly affected	Single dressing ammonium sulphate	0.25 g. boric acid per pot
Badly affected	Double dressing ammonium sulphate	0.5 g. boric acid per pot

As it was impracticable to wash sufficient sand with acid to ensure freedom from boron, a supply was very thoroughly washed with water and dried on glazed cotton sheets in the greenhouse. In case any borax had been used in glazing the sheets, each one was separately tested for boron, only two showing the least suspicion of the element. The sand from these two sheets was reserved for those cultures receiving a heavy dressing of boron. None of the samples of washed sand showed any trace of boron by the turmeric test. The sand for each pot received 3 g. alkaline potassium phosphate and 3.77 g. ammonium sulphate applied in solution and well mixed in before the pot was filled, the boric acid dressing, being added to the solution. The beets were carefully scrubbed to remove as much soil as possible and were then weighed to give a comparative idea of their size at planting, as very great variation occurred in this respect between beets from the same plot. A light watering settled the sand round the roots, and corks and bent glass tubes were inserted into the side drainage holes at the bottom of the pots to prevent loss of fertilizers by leaching and to give a guide as to water-level to avoid over-watering and consequent waterlogging. The pots were randomized, but owing to lack of accommodation it was only possible to duplicate each particular treatment. As, however, the variation in nitrogen dressings proved to have no effect on the incidence of disease, greater replication became available by grouping the nitrogen treatments together.

When transplanting on 4 November care was taken to avoid bruising the large leaves more than necessary, in the hope that the plants would establish themselves more easily with the leaves intact. Actually, how-

ever, the leaves flagged rapidly and did not pick up again, and after some days they were removed. After transplanting the sugar beets were kept under cover in a glasshouse from which frost was excluded, and special attention was given to watering. The growth was so variable that each plant had to be dealt with individually in this respect. After the large outer leaves had died there was a period of no apparent growth while the plants were settling into their new quarters, and then a rosette of fresh leaves began to grow. In normal plants this was succeeded by one central axis, which later developed into a much-branched flowering shoot. In plants which had shown signs of boron deficiency in the field the growing point in the middle of the leafy rosette was killed, thus preventing the growth of a central axis. Instead, a number of secondary shoots appeared round the outer part of the rosette, and these also developed later into flowering shoots. The ultimate behaviour of the shoots was closely related to the treatment of the pots, whereas that of the roots was more influenced by the extent of deficiency trouble when lifted. Typical roots with signs of boron deficiency show a rotted area on the outside of the upper part of the beet, usually associated with a hollow blackened crown (Pl. XXXI, fig. 1). In some cases black streaks appear throughout the tissues in this region, occasionally extending further down (Pl. XXXI, fig. 2).

For the sake of clarity and convenience of reference it seems best to give a classified description of the response of the sugar beet to the various treatments.

A. NO BORON SUPPLIED WHEN TRANSPLANTED

(1) *No original symptoms of boron deficiency*

The morphological feature common to all these plants was the development of a single central stem which, in most cases, proceeded to the flowering stage, and was not killed early and supplanted by numerous secondary shoots. In one case the apex of the main shoot was killed and blackened in the early spring, after which very large axillary shoots developed, becoming blackened at the tips in their turn (Pl. XXXI, fig. 3). With the exception of one plant which soon became unhealthy and died (presumably on account of a diseased root), the rest threw up tall and much-branched flowering stems but, in some cases, the apices and buds showed typical death from boron deficiency by the early summer. Two plants, however, still looked healthy when harvested, in spite of the non-supply of boron. This suggests that neither of these plants had yet exhausted the boron available in its roots, and so had not

reached the stage of exhibiting deficiency symptoms. In this set deficiency symptoms were chiefly shown by the shoots, as the roots were healthy when transplanted and the typical outside blackening and interior decay had not made much progress by the time the experiment concluded.

(2) *Slight original symptoms of boron deficiency*

In all cases the central main stem had died, and a number of smaller shoots developed round the crown to replace it. The response of these shoots to boron deficiency varied according to the condition of the roots as growth continued. In some, the roots resembled those in the previous group, and were relatively clean and healthy. In these plants the effect of boron deficiency was shown by the typical death and blackening of the shoot apices and the flower buds (Pl. XXXII, fig. 4, pot 11), most or all of the shoots being affected. In others the roots were very unhealthy, black and rotten outside at the top, the damage extending downwards and inwards in more severe cases. This was doubtless an extension of the original symptoms of heart-rot which had apparently affected the roots of these plants more seriously. The typical deficiency signs either did not appear or were not very marked in the shoots of these plants, probably because they were weak and late in developing from the rotten roots, and therefore had not reached the stage of exhibiting deficiency.

(3) *Marked original symptoms of boron deficiency*

The primary type of growth was the same as in the slightly affected plants, with a number of lateral shoots from the crown in place of the main axis which was killed by boron deficiency during the first season. One plant made a good start in producing a rosette of leaves, but died later after putting up a solitary secondary shoot. The root proved to be rotten throughout, even the lower part being thoroughly unhealthy. In another plant the shoots looked sickly, but without any blackening and, in all the rest, the typical signs of boron deficiency were manifested in the death and blackening of the stem apices and flowers (Pl. XXXII, fig. 5). The upper parts of all the roots were more or less rotted, the damage being more severe than in those which were only slightly affected in the field. This association of affected shoots and decaying roots doubtless indicates a further stage of deficiency. Where the original trouble was less marked the decayed roots did not bear affected shoots, whereas shoots showing deficiency were sometimes associated with comparatively sound roots.

B. SINGLE DRESSING OF BORIC ACID APPLIED WHEN TRANSPLANTED

(1) *No original symptoms of boron deficiency*

Growth was good in all cases except for one plant which failed to survive transplanting. The central shoot, present in each plant, was occasionally supplemented with a small number of extra shoots, especially in those from the plots receiving a heavy dressing of nitrogen. No sign of boron deficiency appeared in any of the shoots and the roots were good and clean, without the exterior rot so characteristic of those receiving no boron.

(2) *Slight original symptoms of boron deficiency*

In some cases the initial injury was not severe enough to kill the apical meristem, and a weak central shoot emerged in addition to a few secondary ones. Where the central axis was killed the number of secondary shoots ranged up to eleven on a single root. No signs of boron deficiency appeared in the shoots, which flowered freely and looked healthy (Pl. XXXII, fig. 4, pot 30). The roots were very variable, all showing some degree of heart-rot, developed in the field, but this did not extend far down the roots, and apparently had not progressed much, if at all, since transplanting. Two plants showed signs of waterlogging and had rotten tips, but the shoots were not affected.

(3) *Marked original symptoms of boron deficiency*

The light dressing of boric acid applied to the pots was sufficient to prevent deficiency symptoms from appearing in the shoots, but did not enable the plants to recover and make really good growth. No central axes developed, and the number of secondary shoots was less than in the slightly affected plants, the maximum being seven. Most of these were more or less unhealthy in appearance, though no death or blackening of the apices or flowers occurred. Most of the roots were badly affected, apparently due to an extension of the original heart-rot or to subsequent fungal and bacterial attack. Some roots were discoloured the whole way through and only one could be described as fairly healthy, but even that was rotten in the middle of the crown.

C. DOUBLE DRESSING OF BORIC ACID APPLIED WHEN TRANSPLANTED

Quite soon after transplantation it was evident that half the plants in this set were going to fail, as the rosette leaves withered and few or no new ones were produced. These beets died without further development, but all of those which made a satisfactory start grew on steadily.

The failures were not confined to the plants heavily affected with heart-rot, but were equally prevalent in those which were only slightly affected or which showed no symptoms in the field (Table III).

Table III

Number of dead plants out of eighteen in each group

No boron 3	Single boron 4	Double boron 9
No symptoms 6	Slight symptoms 5	Severe symptoms 5
No nitrogen 5	Single nitrogen 6	Double nitrogen 5

There were a certain number of failures with the other pot-culture treatments, but the proportion in this case was so unduly heavy that some contributory cause seems likely. Although no definite proof is forthcoming it may be suggested that the amount of boric acid supplied as a heavy dressing was too great for some of the plants and exercised a toxic effect sufficient to kill them. Where the resistance of individual plants was sufficient to allow them to become properly established this toxic effect did not appear to come into play. It is unlikely that the plants in this set were less strong than the others, as the selection from the field was quite random and the distribution among the treatments was made as evenly as possible. The dead plants were lifted at the same time as others were harvested and were badly decayed all through, probably due to secondary bacterial and fungal infection which found a congenial habitat on the dying or dead roots.

(1) *No original symptoms of boron deficiency*

Each living plant showed a strong central axis and a few secondary shoots all quite healthy and flowering. The roots were good, with no sign of heart-rot, all the crowns being healthy and unaffected.

(2) *Slight original symptoms of boron deficiency*

The secondary shoots replacing the central axis varied in number from four to ten, and were healthy and flowering (Pl. XXXII, fig. 4, pot 50). The roots all showed typical heart-rot in the upper part, being black and somewhat decayed outside, with the characteristic central rot in the crown as well. In one case brown longitudinal specks traversed the whole root but, in others, the lower part was clean and healthy.

(3) *Marked original symptoms of boron deficiency*

From six to twelve side shoots reached the flowering stage, and although in some cases they were not very healthy in appearance, there was no sign of boron deficiency (Pl. XXXII, fig. 6). The general malaise of the aerial shoots was doubtless associated with the unhealthiness of the roots, in which the primary attack of heart-rot had left them susceptible to further rotting. One was unhealthy throughout, and the others showed the typical core of rot in the crown though the lower parts were still fairly sound.

From the above detailed description it is apparent that there was a regular sequence of response correlated with the extent of primary attack and the after-treatment with boron.

(a) When boric acid was omitted from the pot cultures after transplantation typical signs of boron deficiency usually appeared in the shoots regardless of the presence or absence of original symptoms. In the roots there were few signs of deficiency in those plants which were not primarily affected, but in the others the trouble had become more extensive, particularly where the original symptoms were marked.

(b) When boric acid was supplied, either in light or heavy dressing, none of the shoots showed the characteristic death and blackening due to boron deficiency. The roots were good and clean in the absence of primary symptoms, but affected roots did not show recovery. With slight original affection the spread of the trouble was more or less arrested, but with marked deficiency symptoms the progress of decay continued, probably because pathogenic organisms found a favourable habitat and their ravages were not checked by the application of boron.

The differences found between treatments in the fresh weight of the tops (Table IV) were not statistically significant, but they are in concordance with the morphological observations. The weight of tops tended to increase

Table IV

Mean fresh weight of tops, g. per plant

No boron	Single boron	Double boron
49.6	57.6	34.2
No symptoms	Slight symptoms	Severe symptoms
52.4	52.9	36.1
No nitrogen	Single nitrogen	Double nitrogen
47.1	40.0	54.4

S.E. ± 8.5 .

with the first dressing of boron, but the double dressing produced a marked decrease, which is accounted for by the increase in the number of dead plants. The plants showing severe symptoms when taken from the field produced smaller tops than those with none or slight symptoms. This effect was independent of the dead plants, the number of which did not vary between the three groups of none, slight and severe symptoms (Table III). There appeared to be no relation between the weight of tops produced and the original weight of the root.

From the practical point of view the value of these results lies in their possible application to the production of sugar-beet seed. If a crop of beet reserved for seed is affected with heart-rot, it is probable that the trouble will extend during the second year, and that the symptoms will appear in plants hitherto immune. All the affected plants will produce shoots with the characteristic deficiency signs of blackened and dead shoots and flowers and, as these cannot produce fertile seed, the yield will be lowered. If, however, the boron deficiency is remedied by planting in soil containing an adequate supply or by the application of borax before growth again begins, the heart-rot will be controlled and its progress arrested. The plants which came through the first season safely will develop normally, producing healthy shoots and abundant flowers. Also, affected plants will be enabled to develop normal secondary shoots in place of the defunct main axis, showing no signs of boron deficiency in the apices or flowers. Where the initial attack was very severe some of these shoots may be less strong, but they may develop a certain amount of seed instead of being rendered barren by lack of boron.

SUMMARY

1. Heart-rot of sugar beet occurred on experimental plots at Rothamsted during 1935, the severity of the attack decreasing steadily with later sowing, but the effects of spacing of the rows and of treatment with sulphate of ammonia were not significant. Where the number of affected plants per row was high, a higher proportion of affected plants showed severe symptoms.

2. Unaffected sugar beets and others showing slight and severe symptoms of heart-rot were transplanted to sand cultures and treated with light and heavy dressings of boric acid or with none.

3. In the absence of boric acid the characteristic signs of boron deficiency appeared in the shoots, the apices of the stems and the flower buds blackening and dying. This occurred even when no symptoms were present before transplanting.



Fig. 3.



Fig. 1.



Fig. 2.

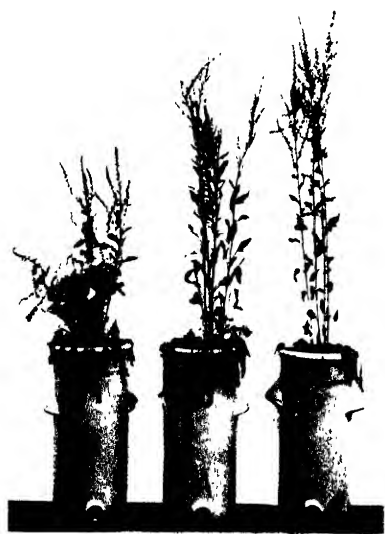


Fig. 4.



Fig. 5.

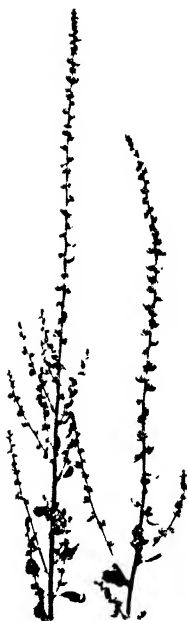


Fig. 6.

4. In the presence of boric acid all plants produced healthy shoots, with no deficiency symptoms. Where heart-rot was originally present and the main axis killed, a number of healthy, lateral shoots were produced.

5. A certain number of plants failed to survive transplanting, the proportion being greatest with the heavy dose of boric acid, with which one-half of the plants died. This suggests a possible toxic action of the heavy dose which did not come into play if the plants were constitutionally able to withstand the initial poisoning and start away into growth.

6. The later addition of boron did not improve the condition of the roots of affected plants, as irremediable damage had been done before transplanting.

7. The possibility of producing healthy shoots in the second year from affected roots is important from the point of view of seed production, as considerable loss may be saved by the use of small amounts of boron compounds.

EXPLANATION OF PLATES XXXI AND XXXII

PLATE XXXI

Fig. 1. Sections of crowns of sugar beet affected by heart-rot.

Fig. 2. Section below crown of affected beet, showing blackened areas.

Fig. 3. Typical death of main axis and increase in secondary shoots, when no boron was supplied after transplanting beets which did not show any original symptoms of heart-rot.

PLATE XXXII

Fig. 4. Plants which showed slight original symptoms of boron deficiency. Pot 11: no boric acid supplied after transplanting. Pot 30: light dressing of boric acid supplied after transplanting. Pot 50: heavy dressing of boric acid supplied after transplanting.

Fig. 5. Plant showing marked boron deficiency in the field and receiving no boric acid after transplanting.

Fig. 6. Plant showing marked boron deficiency in the field, but receiving a heavy dressing of boric acid when transplanted.

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THE ESTIMATION OF LEAF AREA IN FIELD CROPS

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(With One Text-figure)

THE leaf area of a plant is a major determinant of its growth, for the new material produced by the plant in an interval of time is dependent on the size of its assimilating system. West *et al.* (1), and Gregory (2,3) have developed methods of growth analysis, using the rate of increase of dry matter per unit area of leaf ("Unit Leaf Rate" of West *et al.*; "Net Assimilation Rate" of Gregory) as a measure of the balance of the rates of assimilation and respiration. An analysis of plant growth in terms of this function and of the changes with time in leaf area provides more fundamental information than an analysis in terms of the relative growth rate.

In pot culture experiments, particularly in the early stages of growth, it is comparatively easy to measure leaf area directly, because the uniformity of the material allows of the use of a small number of plants. In the later stages, however, when the leaves become numerous and large, measuring the leaf area of every leaf may become extremely laborious. This difficulty is very much intensified in work on field crops. The great variability of the crop necessitates that all observations be made on a number of random samples, each consisting of many plants, in order that the growth changes and the magnitude of the experimental errors may be estimated accurately. The labour of measuring the leaf area of such large samples of plants directly would be impracticably great, for it would involve measuring separately several hundreds, possibly thousands, of leaves at each sampling time.

It is easy, however, to determine the mean leaf weight per plant, by cutting off and weighing the leaves of each sample, and dividing the total leaf weight by the number of plants. A high correlation exists between leaf area and leaf weight, and this fact has been utilized by Ballard & Petrie (1), who have used leaf weight instead of leaf area in calculating unit leaf rate. They point out, however, that other workers have found a drift in the leaf area : leaf weight ratio with time during

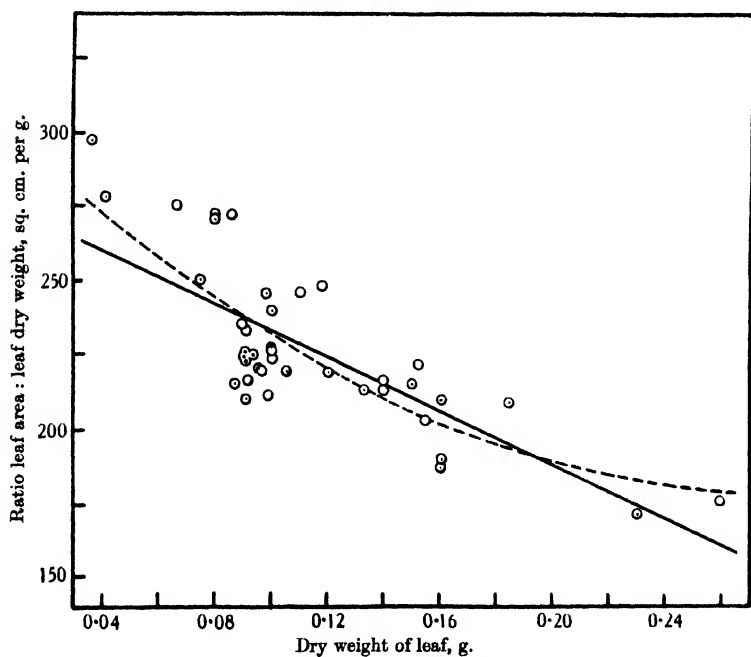
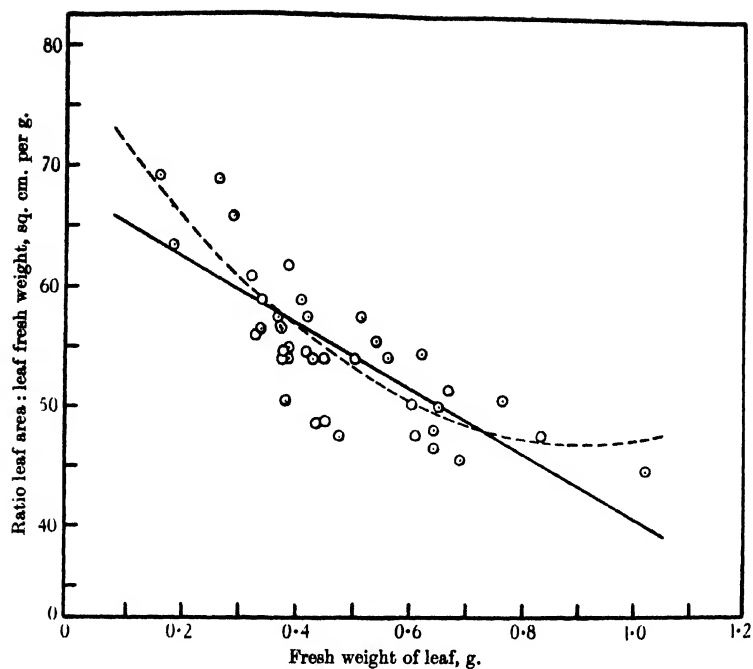


Fig. 1. Relation between leaf area : leaf weight ratio and leaf weight for single wheat leaves. Samples taken on 12th May, 1933.

the growing period, so that the relation between the unit leaf rate, as calculated by them, and as defined by West *et al.*, is not a simple one.

The correlation between leaf area and leaf weight may be made the basis of an indirect method of measuring leaf area. The procedure suggested in this paper for field crops is to estimate mean leaf weight from large random samples of plants, and to determine the leaf area : leaf weight ratio on quite small samples of single leaves. A similar method of estimating grain yield in cereal crops, by sampling for grain : straw ratio and weighing total produce has been suggested by Yates & Zacopanay (5). Gregory (3) has used an indirect method of estimating leaf area on the basis of the leaf area : leaf weight relation, in the later stages of a study of the growth of barley in pot culture, but gives no details of his procedure.

If the leaf area : leaf weight ratio were independent of leaf weight, it would be sufficient to estimate the mean leaf area per plant by multiplying the leaf area : leaf weight ratio, determined on the small sample of leaves, by the mean leaf weight per plant, obtained from the large sampling. On *a priori* grounds, however, it is obvious that the leaf area : leaf weight ratio must decrease with increasing leaf weight, for as the leaf grows in area it also increases in thickness.

Preliminary observations made on wheat leaves showed that there was, in fact, a negative regression of the leaf area : leaf weight ratio on leaf weight. The linear regression was found to account for 55 to 70 per cent of the variance of the ratio, and on fitting a second order term the additional reduction of the variance was small, ranging from 1 to 7 per cent. The nature of the relationship is shown in Fig. 1, where the leaf area per unit weight of leaf is plotted against leaf weight for forty wheat leaves, and the linear and second order regression lines are indicated. A similar type of relation holds whether fresh weight or dry weight is used, and whether single leaves or all the leaves of a shoot taken together are considered. In an experiment carried out on sugar-beet and mangolds, some of the results of which are considered later in this paper, negative linear regressions were found on all occasions, significant in 102 out of a total of 120 samplings. The mean reduction of the variance due to the regression was 71 per cent. It may be concluded that the linear regression is an adequate expression of the relationship between the leaf area : leaf weight ratio and leaf weight, and that the second order regression gives very little additional information to compensate for the large increase in the labour of computation, which fitting the extra term would involve.

METHOD OF ESTIMATING MEAN AREA PER LEAF AND
MEAN LEAF AREA PER PLANT

Let A be the leaf area of a leaf of weight W , and let \bar{A} and \bar{W} be the mean values of A and W for the whole population of N leaves. Assuming that the relation between the leaf area : leaf weight ratio and leaf weight is linear, we have, apart from experimental errors,

$$\frac{A}{W} - \left(\frac{\bar{A}}{\bar{W}} \right) = \beta (W - \bar{W})$$

or $\frac{A}{W} = \kappa + \beta W$, where $\kappa = \left(\frac{\bar{A}}{\bar{W}} \right) - \beta \bar{W}$

or $A = \kappa W + \beta W^2$.

Making a summation over the whole population we have

$$S(A) = \kappa S(W) + \beta S(W^2). \quad \dots\dots(1)$$

Dividing by N we have

$$\bar{A} \text{ (mean area per leaf)} = \kappa \bar{W} + \beta \bar{W}^2. \quad \dots\dots(2)$$

If the mean number of leaves per plant for the whole population is \bar{L} , then the mean leaf area per plant = $\bar{A} \cdot \bar{L} = \bar{L} [\kappa \bar{W} + \beta \bar{W}^2]$. \bar{A} , \bar{W} and \bar{L} are estimated accurately from a large random sampling but not \bar{W}^2 , κ and β , since these involve the determination of individual leaf weights and areas.

Now $S(W^2) = S(W - \bar{W})^2 + N \bar{W}^2$ and substituting in (1) we have

$$S(A) = \kappa S(W) + \beta N \bar{W}^2 + \beta S(W - \bar{W})^2.$$

Dividing by N ,

$$\bar{A} = \kappa \bar{W} + \beta \bar{W}^2 + \frac{\beta S(W - \bar{W})^2}{N}. \quad \dots\dots(3)$$

In addition to the large sample, a small subsample of n leaves is taken, and the area and weight of each leaf is determined. From these areas and weights, k and b , estimates of κ and β respectively are calculated

$(k = \left(\frac{a}{w} \right) - b\bar{w})$, where a and w are areas and weights of leaves of the subsample).

Now it is known that $\frac{1}{n-1} S(w - \bar{w})^2$ taken over the subsample is an unbiased estimate of $\frac{1}{N-1} S(W - \bar{W})^2$ taken over the large sample.

Hence an unbiased estimate of $\frac{1}{N} S(W - \bar{W})^2$ is $\frac{N-1}{N} \cdot \frac{S(w - \bar{w})^2}{n-1}$, and

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since N is large (in the examples which follow $N=500$ to 1000), the factor $\frac{N-1}{N}$ may be replaced by 1.

Substituting in (3), we have

$$A_r \text{ (estimate of } \bar{A}) = k\bar{W} + b\bar{W}^2 + b \frac{S(w-\bar{w})^2}{n-1}, \quad \dots\dots(4)$$

in which \bar{W} is determined from the large sample, and k , b and $\frac{S(w-\bar{w})^2}{n-1}$ are obtained from the subsample.

It is important that the subsample be a strictly random selection from the whole population, in order that $\frac{S(w-\bar{w})^2}{n-1}$ may be an unbiased estimate of $\frac{S(W-\bar{W})^2}{N-1}$. If the subsample were to be used only for the estimation of the regression coefficient b , it would be preferable to select it to give the widest possible range of leaf weight so as to estimate b as accurately as possible, but this is not permissible if the subsample is also used to estimate the variance of mean leaf weight as well as b . A possible alternative method would be to take two subsamples, one selected to give a wide range of leaf weight to be used for the estimation of b , and the other, perhaps somewhat larger, a strictly random selection to provide an estimate of the variance of mean leaf weight. This method might be preferable if considerations of the work involved in measuring the leaf areas require that the subsample on which b is estimated be too small to give an accurate estimate of the variance.

It can be shown that the estimate of \bar{A} obtained by this method is unbiased. From (4) the mean value of A_r from repeated subsamples of n leaves reduces to

$$\begin{aligned} \bar{A} &= \kappa\bar{W} + \beta\bar{W}^2 + \beta\sigma^2, \text{ where } \sigma^2 \text{ is the variance of leaf weight} \\ &= \kappa\bar{W} + \beta\bar{W}^2 + \beta(\bar{W}^2 - \bar{W}^2) \\ &= \kappa\bar{W} + \beta\bar{W}^2, \end{aligned}$$

which is the true value of \bar{A} (expression (2) above).

BIAS IN OTHER METHODS OF ESTIMATING MEAN AREA PER LEAF

The mean area per leaf might be estimated from the product of the mean leaf weight \bar{W} , obtained from the large sample, and the mean

leaf area : leaf weight ratio $\left(\frac{S(a)}{n} \right)$ calculated from the subsample.

It is obvious that the estimate of \bar{A} obtained in this way must be positively biased, since the small leaves, which have a high leaf area : leaf weight ratio, will be overweighted, and the larger leaves, with smaller leaf area : leaf weight ratio, underweighted. The magnitude of the bias can be estimated as follows. For any leaf of the subsample of n leaves

$$\frac{a}{w} = \kappa + \beta w. \text{ Therefore } \overline{\left(\frac{a}{w}\right)} = \kappa + \beta \bar{w}$$

and the estimate of $\bar{A} = \bar{W} \times \overline{\left(\frac{a}{w}\right)} = \bar{W} (\kappa + \beta \bar{w})$.

The average estimate of \bar{A} from repeated subsamples of n leaves

$$\begin{aligned} &= \bar{W} (\kappa + \beta \bar{W}) \\ &= \kappa \bar{W} + \beta \bar{W}^2 - \beta \sigma^2, \text{ since } \sigma^2 = \bar{W}^2 - \bar{W}^2. \end{aligned}$$

The true value of $\bar{A} = \kappa \bar{W} + \beta \bar{W}^2$, so that the bias introduced is $-\beta \sigma^2$, which is positive since β is negative, and independent of the size of the subsample (n).

The objection to this method is partly mitigated if instead of the

unweighted mean leaf area : leaf weight ratio $\frac{S(a)}{n}$, the weighted

mean $\frac{S(a)}{S(w)}$ is used, so that \bar{A} is estimated as $\bar{W} \cdot \frac{S(a)}{S(w)}$. It can be shown, however, that this estimate is also positively biased, though to a less extent than the estimate from the unweighted mean. Thus, for any leaf of the subsample of n leaves

$$a = \kappa w + \beta w^2.$$

Therefore

$$\bar{a} = \kappa \bar{w} + \beta \bar{w}^2.$$

The weighted mean leaf area : leaf weight ratio

$$\frac{S(a)}{S(w)} = \frac{\bar{a}}{\bar{w}} = \kappa + \beta \frac{\bar{w}^2}{\bar{w}},$$

and the estimate of $\bar{A} = \bar{W} \times \frac{\bar{a}}{\bar{w}} = \kappa \bar{W} + \beta \frac{\bar{w}^2}{\bar{w}} \cdot \bar{W}$.

The mean value of the estimate of \bar{A} from repeated subsamples of n leaves

$$\begin{aligned} &= \kappa \bar{W} + \beta \bar{W} \left[\text{mean value of } \frac{\bar{w}^2}{\bar{w}} \right] \\ &= \kappa \bar{W} + \beta \bar{W} \left[\text{mean value of } \bar{w} + \frac{S(w - \bar{w})^2}{n \bar{w}} \right] \quad \dots (5) \end{aligned}$$

$$\begin{aligned}
 (\text{since } \overline{w^2} &= \frac{S(w^2)}{n\bar{w}} = \frac{S(w - \bar{w})^2 + n\bar{w}^2}{n\bar{w}} = \frac{S(w - \bar{w})^2}{n\bar{w}} + \bar{w}) \\
 &= \kappa \bar{W} + \beta \bar{W} \left(\bar{W} + \frac{n-1}{n} \cdot \frac{\sigma^2}{\bar{W}} \right) \text{ approximately,} \\
 &= \kappa \bar{W} + \beta (\bar{W}^2 + \sigma^2) - \frac{1}{n} \beta \sigma^2 \\
 &+ \kappa \bar{W} + \beta \bar{W}^2 - \frac{1}{n} \beta \sigma^2.
 \end{aligned}$$

The bias introduced is therefore $-\frac{1}{n} \beta \sigma^2$, or $1/n$ th of the bias in the estimate from the unweighted mean. It is dependent on the size of the subsample, becoming zero when n is very large, as obviously it must, since for the whole population $\bar{W} \cdot \frac{\bar{A}}{\bar{W}}$ is equal to \bar{A} . It should be pointed out that these estimates of the bias are correct only if the subsample is a random selection from the whole population, so that the mean of \bar{w} tends to \bar{W} in repeated subsamples of n leaves, and the estimate of σ^2 , the variance of mean leaf weight, is unbiased.

EXPERIMENTAL RESULTS

A sampling experiment was carried out on sugar-beet and mangolds in 1934, in which growth observations were made by sampling at fortnightly intervals. The experiment consisted of six blocks, each of two plots, one of sugar-beet and one of mangolds, and the blocks were sown singly at successive intervals of a fortnight. Sampling was begun when the crops were thinned and ten complete samplings were carried out after the thinning of the last sown plots. On each occasion a random sample of twenty plants was taken from each plot, the number of leaves in the whole sample was counted, and the total fresh weight of leaf lamina determined. A random subsample of ten leaves was taken, one from each of ten plants selected at random from the twenty plants of a sample. The lamina was cut off and weighed and its area measured by printing on "blue-print" paper, cutting out the print and weighing it. The mean leaf area per plant was calculated for each sample by the three methods which have been discussed. The estimates obtained by the unweighted mean leaf area: leaf weight ratio method were consistently greater than those calculated by the regression method. The estimates from the weighted mean showed a similar but smaller positive bias. The mean values for the sixty samplings of sugar-beet and of mangolds are shown in Table I.

Table I. *Mean leaf area per plant, sq. dm. (mean of sixty samplings)*

Method of estimation	Sugar-beet	Mangolds
Unweighted mean	51.41	40.89
Weighted mean	40.81	30.49
Regression	38.03	28.97
Bias:		
Unweighted mean	13.38	11.92
Weighted mean	2.78	1.52
Ratio unweighted : weighted	4.8	7.8

It has been shown theoretically that the bias in the unweighted mean estimate should be n times the bias in the weighted mean estimate, where n is the number of leaves in the subsample. In this experiment $n=10$, and the ratio of the biases was found to be 4.8 for sugar-beet and 7.8 for mangolds. The latter figure is in fair agreement with theory, but the discrepancy for sugar-beet is more serious. The most probable explanation is that the estimate of mean leaf weight in the subsample was positively biased. In the sugar-beet there was a considerable development of axillary buds, forming many leaves of small area. In selecting the subsample of ten leaves, these were ignored, as it was difficult to devise any simple method of strict random selection which would include them, but they were included in the estimate of mean leaf weight in the large samples (W). The leaves on the main axis were selected by counting back from the youngest leaf in the order of production, until a leaf of a number selected from a table of random numbers was reached. In the mangolds, however, axillary leaves were rare. If \bar{w} is an unbiased estimate of \bar{W} , it would be expected that in a series of samples, the number of occasions on which \bar{w} exceeded \bar{W} would be equal to the number on which \bar{w} was less than \bar{W} . This was found to be true approximately for the mangolds, but for sugar-beet the number of occasions on which \bar{W} was greater than \bar{w} was markedly in excess of expectation. This is shown in Table II.

Table II

	Sugar-beet	Mangolds
Number of occasions on which \bar{w} was greater than \bar{W}	40	28
Number of occasions on which w was less than \bar{W}	20	32

This bias in the estimate of \bar{w} may have affected the estimates of mean leaf area by the regression method, for though k and b will not have been affected, the estimate of σ^2 may be biased.

The estimates of mean leaf area per plant calculated by the unweighted mean method were considerably more variable than estimates

by the weighted mean, or regression methods. Table III shows the residual variances, after elimination of variance due to plots, times of sampling and the interactions with time of the linear regressions on sowing date.

Table III. *Residual variance of mean leaf area per plant (72 D.F.)*

Unweighted mean method	74.89
Weighted mean method	35.11
Regression method	48.14

The variances of the estimates calculated by the weighted mean and regression methods did not differ significantly.

A similar method of estimating mean leaf area per plant and per metre row of crop has been employed for wheat. The labour of cutting off and weighing the leaves from a large number of random samples, in order to determine the mean leaf weight \bar{W} , was found to be impracticably great, and to avoid this, the estimates were based on determinations of the leaf area : plant dry weight ratio and its linear regression on plant dry weight. The method of calculation was exactly similar to that described above, where the leaf area : leaf weight ratio and its regression on leaf weight were used. Significant negative regressions of the leaf area : plant dry weight ratio were found during the period from the beginning of May onwards, but during the earlier stages of growth the regression coefficients were small and not significant. Positive and negative values of the coefficient were equally frequent, indicating that during this period of growth the leaf area : plant dry weight ratio was independent of plant dry weight. The regression of the leaf area : leaf dry weight ratio on leaf dry weight was found to be consistently negative and significant on almost all occasions, as in the sugar-beet and mangold data.

SUMMARY

1. It is shown that the leaf area : leaf weight ratio decreases with increasing leaf weight.
2. The relation between the leaf area : leaf weight ratio and leaf weight is well fitted by a linear regression equation.
3. A method of estimating the mean leaf area per leaf or per plant of a field crop by means of this regression is described. The mean weight per leaf is determined by a large sampling, and the leaf area : leaf weight ratio and its regression on leaf weight are estimated on a small subsidiary sample.

4. Alternative methods of estimation from the mean leaf weight and either the unweighted or the weighted mean leaf area : leaf weight ratio are shown to give positively biased estimates of mean leaf area.

5. It is emphasized that the small sample, from which the leaf area : leaf weight ratio and its regression on leaf weight are determined, must be a strictly random selection from the whole population.

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THE EARLY DEVELOPMENT OF THE COTTON FIBRE

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INTRODUCTION.—The development of the cotton hair is obviously a subject of considerable economic importance, and it is not surprising therefore that it has received increasing attention during recent years. Nevertheless, considerable discrepancy and confusion appear to exist in the interpretation of results. It seemed possible that a detailed cytological study of diverse material would yield some explanation of these discrepancies. For this purpose, material of Old and New World varieties of cotton obtained from India and Egypt was compared with cotton grown under glass in England.

Over fifty years ago, Bowman (1882) first discussed the formation of cotton hairs, concluding that they originated "in the second layer of cells beneath the cuticle of the seed." Many years later, Balls (1915) published an outline account of the development of all tissues of the cotton seed after fertilization. He found that the fibres begin to grow out from the epidermal cells of the ovule on the day that the flower opens, and that "there does not appear to be any further growth of epidermal cells into lint hairs after this first day, in spite of accepted statements to the contrary." Later (1919) he suggested that "possibly irregularities in length may arise from distribution of the normal simultaneous 'sprouting' of these cells over several days."

Turner (1929) contested this view, pointing out that on Balls' conception the number of hairs per seed must remain constant, and that the number of fibres per unit area of seed surface must decrease as the seed increases in volume. He suggested that the differentiation of hairs continues for some time after flowering. Gulati (1930) found that there is a sevenfold increase in the number of hairs in the first five days after flowering. This continued differentiation of hairs must be resultant upon continued mitotic divisions in the epidermal layers of the outer integument. Gulati claimed that mitosis occurs in these cells up to the tenth day after flowering. Singh (1931) made certain cytological observations and supported the view that the number of hairs increases during the life history of the ovule. Farr (1933)

observed mitosis in the epidermis up to the twelfth day after flowering. It is unfortunate that none of these cytological observations are supported by adequate photomicrographs of mitosis in the epidermis after the first few days, for from the photographs published by Barritt (1932) it would seem that division in the epidermis must have ceased long before even the seventh day after flowering. Ayyar and Ayyangar (1933) consider that the epidermal cells remain in a young growing condition for some time after flowering, stating that "we did not detect (in sections of Cambodia cotton ovules) actual mitosis in the epidermal cells beyond the second day after flower opening, but the nucleus was quite prominent in them till they were twenty days old." These authors also revert to the suggested subepidermal origin of the fibres.

It will be shown that great variation occurs in the development of the tissues of different seeds within a single boll and that different parts of a single seed are often differently developed. Thus it will be found possible to reconcile several of the contradictory statements outlined above.

MATERIAL.—One dozen plants of the variety Sakel were grown in a chamber of a glasshouse at Rothamsted Experimental Station in the summer of 1933. Seed from 1931 plants was obtained from Professor R. H. Stoughton. It was sown on February 15 and flowering commenced towards the end of May. Cotton had been grown in this same glasshouse for several seasons, so that information as to the best conditions was available. It was of course not possible to obtain the normal growing conditions of the cotton plant in tropical and sub-tropical regions. The light intensity in this country is very much lower, but Balls has shown that the too great light intensity experienced in Egypt retards growth. In England, the maximum intensity is probably about the optimum for growth, and it is during the dull periods that growth is retarded. Over the two months of June and July when all fixing was done, the average maximum day temperature was 39.5° C. and the average minimum was 21.4° C. The glasshouse chamber faced westwards and was in direct sunlight during the afternoon, so that the highest temperatures were attained about 3 to 4 p.m., the lowest being experienced about 3 to 4 a.m. Possibly the temperatures are lower than the optimum, but their retarding effect seems to be counterbalanced by other factors—the relatively long day experienced in this country in summer and the fact that the greater degree of humidity of the atmosphere of a glasshouse prevents wilting which may occur in the field. For the purposes of this paper it was essential that the flowers should develop

normally after fertilization. The time required after pollination for the boll to ripen affords a criterion of the normality of seed development. The bolls of these plants ripened 50 days after the flowers opened, the normal time of ripening varying from 40 to 60 days (Balls, 1915).

For comparison fixed material was obtained from Indore in Central India and also from Egypt. The material from Indore consisted of a New World type (Indore No. 1, a selection from Cambodia, of the *Gossypium hirsutum* type) and of an Asiatic type (Malvi, a selection from the local cotton of Indore. According to Gammie it is *G. neglectum* var. *malvensis*, whilst Harland classified it as *G. arboreum* L. var. *Nanking*). Samples of Maarad cotton were obtained from Egypt. Unless special mention is made the general description which will follow can be regarded as applicable to all the varieties examined.

METHODS.—On the day each flower opened it was labelled with a tab bearing the date and an appropriate number. In a few cases flowers were cross-pollinated, but usually they were allowed to become self-pollinated, pollination occurring naturally within a few hours of the opening of the flower.

A number of trials were made to find a suitable fixative. Among those tried were Carnoy's, Flemming's medium with or without acetic acid, Champy's and Helly's fluids and a chrome-acetic solution. Allen's modification of Bouin's fluid (B-15) was found to give excellent fixation in the early stages of seed development. Satisfactory fixation in the later stages is exceedingly difficult to obtain; the results yielded by the latter fixative were better than any given by other solutions. Much of the material grown at Rothamsted and all of that obtained from India and Egypt was therefore fixed in Allen's Bouin.

In England the material was fixed within one hour of noon (*i.e.*, between 11 a.m. and 1 p.m. Greenwich Mean Time), when maximum cell division is generally supposed to occur in this climate. The Indian material was fixed about 10 to 11 a.m. and that from Egypt between 9 a.m. and noon or 3 and 5 p.m. Bolls up to seven days old were cut into quadrants before fixing; after seven days, the seeds were dissected out before fixing. The fixative was warmed to 38° C. and an exhaust pump was always used. The material was left in the fixative for 4 to 6 hours. It was then passed rapidly through graded alcohols to 70 per cent. alcohol saturated with lithium carbonate. This solution was poured off and renewed at intervals until all the picric acid was washed out from the seeds. (Material from India and Egypt travelled in this solution.) Dehydration was then

completed with alcohol, the material was cleared in cedarwood oil and was embedded in paraffin wax of melting-point 52° or 58° C. according to the age and consequent hardness of the seeds. Sections were cut at 6 to 15μ .

A variety of stains were used. The iodine-gentian-violet method with or without a counterstain gave excellent results.

THE EPIDERMIS OF THE RESTING OVULE.—In the early part of the day on which the flower opens and pollen is transferred to the stigma, although occasional mitoses may be found, relatively little nuclear activity is observed in the outer epidermis of the outer integument of the ovule. This layer now consists essentially of closely packed cuboidal cells, radially elongated and each containing a large nucleus and richly staining cytoplasm (Fig. 1). Occasional cells bulge slightly to the exterior. The latter are already differentiated and will give rise to the first formed hairs without undergoing any further division. A few stomata are already differentiated (Fig. 2).

DIFFERENTIATION OF THE EPIDERMIS OF THE SEED COAT.—After pollination, the epidermis enters a phase of great activity. The undifferentiated cells divide, and certain of the daughter cells may develop into hairs or stomata. The other daughter cells divide repeatedly until all in turn are differentiated as lint hairs, as fuzz hairs, as stomata, or in such a way as to become coloured and thickened to form the protective epidermis of the mature seed.

Twenty-four hours after pollination the primordial hair cells distinguishable on the previous day have elongated radially to several times their original size (Fig. 3). As each cell elongates, the nucleus passes outwards so that it remains always about the centre of the cell. A few stomata are also differentiated on the first day; these and the young hairs are usually confined to the chalazal end of the seed. In the case of the Maarad cotton occasional seeds show hairs developing first at the micropylar end. The undifferentiated epidermal cells are in a state of very active division. The pollen tube reaches the embryo sac and fertilization occurs between one and two days after pollination (Fig. 5).

Singh (1931) stated that in bolls "forty-eight hours old almost all the epidermal cells have been found to be involved in the formation of fibres." This is true only of certain areas of the seed surface. At the chalazal end (Figs. 4 and 5) a very large proportion of the cells are developing into hairs, but towards the micropylar end the vast majority of the cells are usually undifferentiated at this time. As the hairs at the chalazal end are so closely packed it is obvious

that to produce the spaced hairs of the mature seed the few epidermal cells remaining amongst them must divide without the daughter cells always giving rise to further hairs.

The undifferentiated cells near the micropyle continue to divide rapidly. Actual counts were not made, but the general impression given by the preparations is to the effect that the number of mitoses reaches a peak about the second day after pollination or very soon after fertilization.

As the cells become differentiated into hairs or stomata, they obviously lose their power of division, and as more and more cells are differentiated, the number in mitosis at one time must decrease. At the third day, mitosis is still of quite frequent occurrence; on the fourth and fifth days, groups of meristematic epidermal cells (Fig. 7), as well as isolated dividing cells amongst long hairs (Fig. 6), are found in every seed. Hairs of various lengths occur in every section (Figs. 10 to 14) and stomata are well developed, especially towards the chalazal end. About this time certain areas of the epidermis are seen to have lost all power of division. The undifferentiated cells increase in volume, the nucleus remaining small and entering a resting condition. The cell vacuole becomes filled with the golden coloured ~~cytochrome~~^{protein}, and no cell in which ~~cytochrome~~^{protein} has developed is ever seen to divide.

Young hairs now begin to arise from the cells at the micropylar end of the seed and a few are formed also between the now much elongated hair cells towards the chalazal end. When they are about two days old, ~~cytochrome is formed~~^{protein is formed} in a number of these, suggesting that many of the later formed fibres go to constitute the "fuzz." This is indicated also by their position: a tuft of fuzz hairs is usually present around the micropyle of the mature seed, and patches of fuzz hair occur also amongst the longer lint hairs towards the chalazal end. However, these later formed hairs are not all of the fuzz type. Lint and fuzz hairs are eventually found intermingled in those areas where no hairs are developed until several days after pollination. The diameter of these younger lint hairs seems to be slightly greater than that of the earlier formed fibres.

About six days after pollination, mitosis becomes increasingly difficult to locate in the epidermis. The latest dates after flowering at which it was observed are:

Sakel	Up to 10 days (Fig. 17).
Indore No. 1.	Up to 9 days.
Malvi	Up to 8 days (Fig. 10).
Maarad	Up to 9 days (Fig 16).

It is probable that mitoses do occur at still later dates. Divisions were not actually observed later, although uncoloured cells with large nuclei apparently still capable of division were found up to twelve days. By the twelfth day, except in these rare instances, all the epidermal cells are filled with ~~cytochrome~~^{cytogenin}, as are also the fuzz hairs, only the lint hairs and the stomata remaining colourless. In occasional seeds this condition is reached by the seventh day, and frequently it is attained by the tenth day after flowering.

The intermingling of fully differentiated and meristematic cells that occurs in the epidermis of the cotton seed is most unusual. In the case of cambium and of the primary meristems, the cells remain quite undifferentiated until all in the immediate neighbourhood have ceased to divide.

VARIABILITY.—Previous writers have tended to stress the latest date on which they were able to observe mitosis. Very little significance should be attached to the exact time of cessation of all mitosis. This date is difficult or almost impossible to determine, and slight variations in conditions of growth may bring about differences in the rapidity of cell division. It is the variability in the growth of the fibres that should be emphasised. The foregoing description has shown that there is great variation in the development of different regions of the seed coat. Generally development is earlier towards the chalazal end of the seed (Figs. 5 and 14). This is shown not only in the epidermis but also in the internal tissues such as the palisade layer. The results described are in harmony with those of Koshal and Ahmad (1932), who found considerable differences in the physical properties of the fibres from different parts of the seed coat. They found the mean fibre length for the fibres at the base of the seed to be significantly greater than for fibres at the apex of the seed. That the basal fibres should be longer than the apical ones is in accordance with the fact that the basal ones are several days older than the apical, and that the mean fibre weight per unit length and the mean fibre strength should be greater for the apical fibres is compatible with the slightly greater diameter of the apical fibres.

Great variation also exists from seed to seed within the same boll. This was realized by Bailey and has led to the custom of selecting always the second seed from the base when sampling. It is also well exemplified by two photographs (Figs. 19 and 20) taken from two seeds of a single boll which were fixed, embedded and sectioned together and mounted on the same slide.

The amount of variation occurring from cell to cell of a single

seed and from seed to seed within a single boll is greater than the differences in development between varieties or even between Old and New World types of cotton. Obviously from an economic standpoint this variation is most undesirable, but its existence cannot be too greatly stressed. Failure to realize this variability, whilst drawing conclusions and basing hypotheses on the cursory examination of hastily prepared sections of a few seeds, has led to infinite confusion. The sectioning of a whole seed is a long and tedious process, but unless this is done it is impossible to determine whether cell division is complete, as whilst some areas are fully differentiated, others may remain meristematic. Even if whole seeds are sectioned mitoses may still be missed owing to the material having been fixed at an unfavourable period of the day,* although with experience cells still capable of division can usually be recognized. Special emphasis is laid on this variability, for not only has it led to confusion in the interpretation of cytological preparations, but such differences in the early development of the fibres cannot fail to affect the uniformity of the mature lint.

THE NUCLEUS OF THE PRIMORDIAL HAIR CELL.—Ayyar and Ayyangar (1932) noticed that the nuclei of the primordial lint hairs increase in size and that several small nucleoli appear; the latter fuse to form a single nucleolus. Barritt (1933) has pointed out that in the normal course of mitosis the nucleolus disappears during the prophase. At the telophase one or more nucleoli arise *de novo*. If several such nucleoli appear they may later fuse to form one body; this proved to be the case in cotton, and is not confined to the primordial hair cells. After each mitotic division several small nucleoli arise in each daughter cell. If the daughter cell is to give rise to a hair the nucleus increases in size, and the nucleoli fuse into one large spherical body which later often contains a crystal. If the daughter cell remains undifferentiated, these small nucleoli tend to fuse together, but a new mitotic division may be commenced before fusion occurs. In the case of daughter cells which have ceased to divide, and have assumed the characters of mature epidermal cells, the nucleoli generally fuse together into one body.

THE HAIR BASE.—Barritt (1929) has claimed that the portion of the hair embedded in the epidermis does not grow at all, and as

* In this connection it is interesting to note that many more cells were actually seen in the processes of cytokinesis in the material grown in England and in Egypt than were found in the two types sent from India. As the development of all varieties appeared to be as nearly parallel as is possible in such inconstant material, presumably the times of day selected for fixing in England and in Egypt were about the times of maximum division, but the time of fixation in India was not so fortunately chosen.

the neighbouring cells increase in size the hair base becomes crushed. On this observation he based his theory of fibre nutrition from the "boll-sap." Farr (1931) has denied the existence of the constricted hair base, stating that the apparent flattening is due to the plane in which the section is cut. The writer finds that for four or five days the bases of the hair cells do increase in size, keeping pace with the diameter of the fibre (Fig. 13). After this there appears to be no further increase and the bases may become constricted by pressure from the neighbouring epidermal cells which are increasing in size and in number. That the constriction is real can be determined either by focussing or by examination of adjacent sections. If the constriction were merely due to the plane of section, then the full width of the hair base should be so revealed, but such was not found to be the case.

The material grown in England was the only fresh material available which could be examined for "boll-sap." No liquid was found in the boll cavity. The question would bear further examination as the function of the stomata (Fig. 15) on the seed coat remains obscure.

STOMATA AND THE FUZZ HAIRS.—Ayyar and Ayyangar (1933) reverted to the theory of Bowman (1882) that the hairs are of subepidermal origin, making the naive suggestion that one of the subepidermal cells opposite a stoma "enlarges centrifugally and gradually finds its way out between the guard cells." These hairs are thought to be fuzz hairs, and in this way the constriction of the hair base is accounted for. Now, in the young seed all the tissues including the epidermis, hairs and stomata are colourless. As the seed ripens, the epidermal cells and the fuzz hairs become filled with ~~cytoplasm~~ ^{chromatin}, but the guard cells of the stomata remain always colourless and their contents are unaffected by all the more usual dyes. If then a hair were to grow through the stomatal cavity we should expect to see a colourless cell on each side of the hair. This is not shown in Ayyar and Ayyangar's photographs where all the epidermal cells are densely coloured. Nor could it be observed in any sections or epidermal strippings from the mature seed (Fig. 21).

SUMMARY.—The development of the cotton fibres from primordial cells in the epidermis is described. Pollination stimulates cell division in the epidermal layer. Mitosis continues in the epidermis and young hairs are differentiated for a considerable period after flowering. The number of epidermal cells in active division seems to reach a peak at about the second day, but mitosis occurs to a diminishing extent until at least ten days after flowering.

The discrepancies in the results of previous workers appear to be due to an unexpected variation in the rate of development and differentiation in different cells of the same seeds, and in different seeds within the same boll.

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EXPLANATION OF PLATES.

All photomicrographs were taken with a Leitz "Makam" camera. A Leitz "Monla" lamp, suitably screened with a Wratten "M" colour filter, was used as the source of illumination.

- FIG. 1.—Sakel. Day of pollination. The epidermis of the seed shows occasional cells in mitosis. 900 \times .
- FIG. 2.—Sakel. Day of pollination. Some stomata are already differentiated. 900 \times .
- FIG. 3.—Sakel. One day. Mitosis occurs in the epidermis and young hairs are differentiated. 900 \times .
- FIG. 4.—Sakel. Two days. Towards the chalazal end of the seed, a very large proportion of the epidermal cells are differentiated as hairs. 140 \times .
- FIG. 5.—Sakel. Two days. Longitudinal sections of the seeds show young hairs developing around the chalazal end. Very few hairs are seen towards the micropylar end. The pollen tube has reached the embryo sac. 30 \times .
- FIG. 6.—Sakel. Four days. Mitosis occurs in the epidermal cells between the hair bases. 900 \times .
- FIG. 7.—Sakel. Five days. Portions of the epidermis remain meristematic. 900 \times .
- FIG. 8.—Maarad. Seven days. Mitosis continues in the epidermis. 900 \times .
- FIG. 9.—Indore No. 1. Seven days. Mitosis still continues in the epidermal cells between the hair bases which are becoming constricted. 900 \times .
- FIG. 10.—Malvi. Eight days. An area of the epidermis near the micropyle which has remained in a meristematic condition. Young hairs are being differentiated. Sections of older hairs are also seen in the background. 900 \times .
- FIG. 11.—Sakel. Seven days. Very young fibres are growing amongst older ones. 140 \times .
- FIG. 12.—Maarad. Seven days. Hairs one or two days old are growing amongst older fibres. 140 \times .
- FIG. 13.—Maarad. Seven days. Constricted hair bases are seen between the epidermal cells. 140 \times .
- FIG. 14.—Sakel. Eight days. Longitudinal section of a seed shows long hairs and short hairs projecting from the epidermis and also a region of the epidermis which has not yet given rise to hairs. 20 \times .
- FIG. 15.—Maarad. Eight days. Section through the epidermis shows a stoma and a hair base. 900 \times .
- FIG. 16.—Maarad. Nine days. A region of the epidermis still remains meristematic. 900 \times .
- FIG. 17.—Sakel. Ten days. Mitosis may still occur in the epidermis. 900 \times .
- FIG. 18.—Sakel. Nine days. Mitosis occurs in the epidermis. Sections of long hairs are also seen. 450 \times .
- FIGS. 19 and 20.—Sakel. Ten days. These two sections were made from two different seeds from the same boll. In one the epidermis is meristematic; in the other, mitosis has long ceased. 450 \times .
- FIG. 21.—Sakel. Fourteen days. The hair base, constricted between the epidermal cells, projects into the subepidermal layer. 900 \times .

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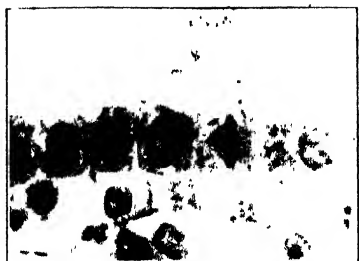


FIG. 1.

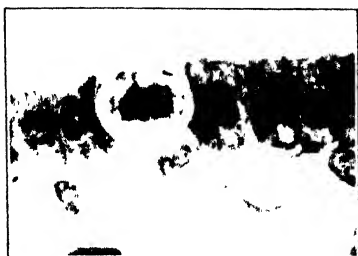


FIG. 2.

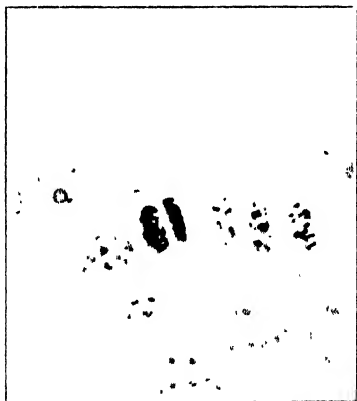


FIG. 3.

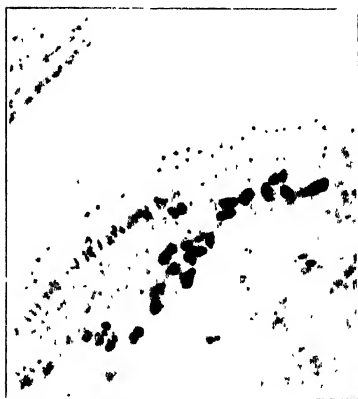


FIG. 4.



FIG. 5.

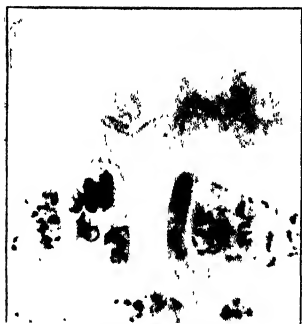


FIG. 6.

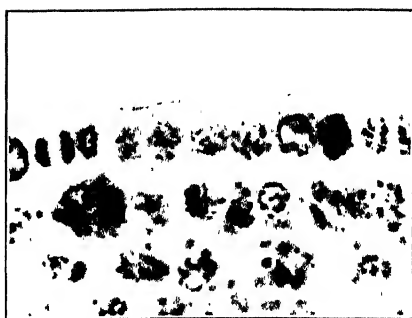


FIG. 7.

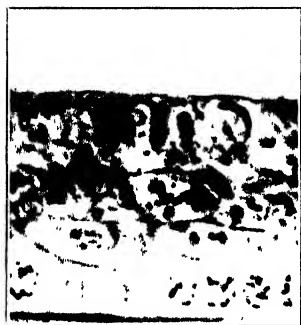


FIG. 8.

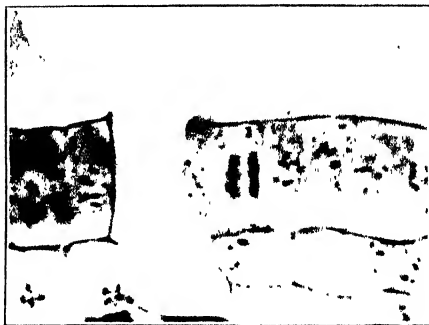


FIG. 9



FIG. 10.



FIG. 11



FIG. 12



FIG. 13

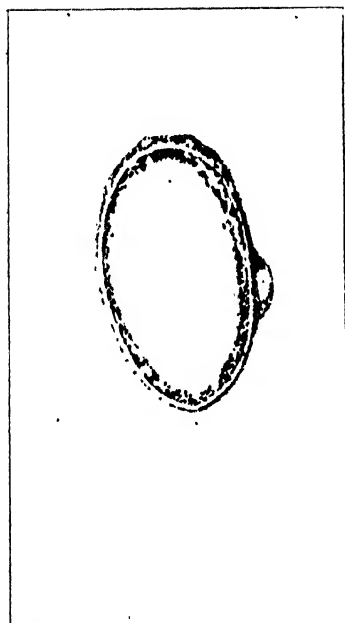


FIG. 14.



FIG. 15.



FIG. 16

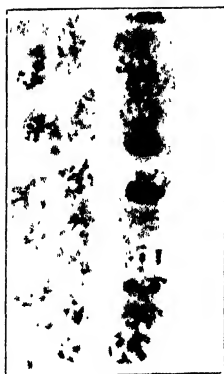


FIG. 17.

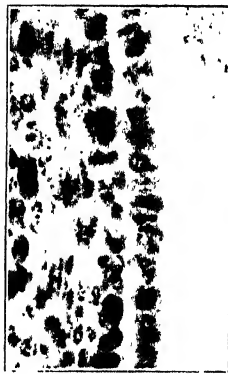


FIG. 18.

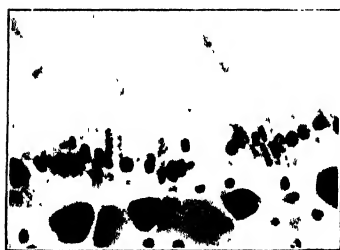


FIG. 19.



FIG. 20.



FIG. 21.

Observations on the Oxygen Uptake of Isolated Plant Tissue

I. The Effect of Phosphate and of Added Carbohydrate

BY

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AND

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With one Figure in the Text.

INTRODUCTION

IN the course of an examination of the possibility of oxygen uptake by the agent of aucuba virus disease of tomato, a number of studies of the behaviour of plant tissue in Barcroft respirometers were made. The experiments with the virus material yielded negative results; in no instance, with crude virus juice filtered through Pasteur-Chamberland L3 candles, or with purified and concentrated virus material (Caldwell, 1935), was there any indication that oxygen was taken up, even when large quantities of active virus-agent were present in the experimental material.

Subsequent experiments, performed with tissue of healthy and diseased plants, while they threw little light on the problem of virus disease, yet gave results which are suggestive of other lines of investigation, and some of these results are recorded in this paper.

MATERIALS AND METHODS

The material used was slices of tomato stem tissue prepared by removing, under sterile conditions, the extra-cambial tissues and then cutting the remaining tissue longitudinally into slices about 2.5 cm. long by 0.5 mm. thick. In preliminary experiments it was found that, if thicker blocks of tissue were used, the oxygen uptake was limited by the rate of diffusion into the block; the type of results obtained are illustrated in Table I.

As filling the Barcroft bottles with oxygen instead of air (to increase the external oxygen concentration) caused no measurable increase in the oxygen uptake of slices 0.5 mm. thick, it was concluded that the slices were sufficiently thin to allow of adequate diffusion of oxygen into the interior of the tissue (cf. Warburg, 1930).

The apparatus used for the measurement of oxygen uptake consisted of Barcroft differential manometers; the customary technique was employed (Dixon, 1934). All the measurements were made at 26° C. The oxygen uptake is expressed throughout as $\mu\text{l.}$ of gas (dry at N.T.P.) taken up per hour, per gramme fresh weight of tissue.

TABLE I
Comparison of Blocks and Slices

Method of cutting.	Tissue suspended in.	Oxygen uptake ($\mu\text{l.}/\text{gm.}/\text{hr.}$)		
		(1)	(2)	(3)
Whole block.	M/10 KH_2PO_4	64	56	61
Half blocks	"	73	74	78
Slices	"	81	78	84
Whole block.	M/30 KH_2PO_4	—	63	—
	M/30 $\text{KH}_2\text{PO}_4 + 0.5$ per cent. glucose	—	70	—
Half blocks	M/30 KH_2PO_4	—	62	—
	M/30 $\text{KH}_2\text{PO}_4 + 0.5$ per cent. glucose	—	79	—
Slices	M/30 KH_2PO_4	—	70	—
	M/30 $\text{KH}_2\text{PO}_4 + 0.5$ per cent. glucose	—	88	—

The procedure in a typical experiment was as follows: the slices of tomato stem, prepared as described above, were divided into six approximately equal batches. Each batch (weighing between 0.1 gm. and 0.7 gm.) was placed in a tared Barcroft bottle containing 3 ml. of sterile dilute potassium dihydrogen phosphate (KH_2PO_4) solution, and the whole weighed. The usual roll of filter-paper and KOH solution were added, the apparatus placed in the bath and brought to equilibrium, and readings were then taken at intervals over a period of six hours. Dilute acid potassium phosphate was used in which to suspend the slices as it is a non-poisonous medium of an acidity comparable with that of tomato juice. In the earlier experiments the concentration of phosphate was varied; in the later experiments, only one concentration of phosphate (M/30) was used, but sugar was added to some of the bottles, the required amount being dissolved beforehand in the phosphate medium.

In all the work recorded in this paper precautions were taken to ensure that no bacterial contamination took place; when on one occasion this did occur the increase in oxygen uptake late in the six-hour period was so marked that it was easy to see that something unusual had happened.

Note on the accuracy of the results.

Every figure for oxygen uptake given in the tables is a mean value, derived from six samples in Tables II and III, and two or three samples in the later tables. The actual results from which the means are taken varied over a fairly wide range. The main factor contributing to this variation was probably the difficulty of obtaining uniform sets of samples of the relatively large size necessitated by the small oxygen

uptake of plant tissue. Where the mean oxygen uptake was above $40 \mu\text{l./gm./hr.}$, the average standard deviation was found to be about 10 per cent. of the mean. It follows that differences, due to treatment, of less than 10 per cent. of the mean value of the control cannot be detected. Most of the differences reported are of a higher order; significance tests were applied to them all, and those differences which were found not to be significant are so marked in the tables.

RESULTS

1. Phosphate concentration and age of tissue.

In the first series of experiments tissue from healthy plants was used; the plants were of two different sizes; plants in the 5th leaf stage (referred to as 'very young' plants), and plants in the 6th to 12th leaf stage (referred to as 'young' plants). The oxygen uptake of tissue from plants of both types was measured in distilled water, and in different concentrations of phosphate. The results are given in Table II.

TABLE II
Oxygen Uptake of Slices of Tomato Stem (Healthy Plants)

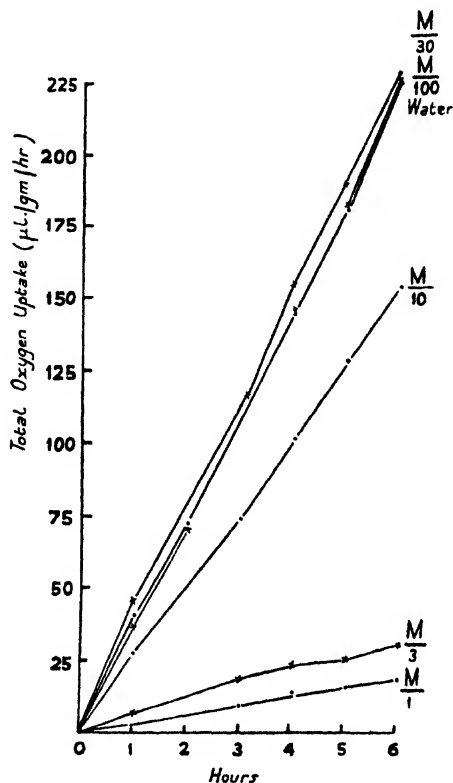
Concentration of KH_2PO_4 .	Mean oxygen uptake ($\mu\text{l./gm./hr.}$).	
	Very young plants.	Young plants.
M	8.1	—
M/3	14.0	39.5
M/10	59.5	114.2
M/30	87.8	137.4
M/100	86.0	112.3
Water	85.9	114.5

It will be observed that the level of oxygen uptake is consistently higher in the tissue derived from young, as compared with very young, plants, and also that the oxygen uptake is depressed in concentrations of phosphate stronger than M/30.

[An examination of the data from which the mean values were derived showed that (a) the mean value for oxygen uptake at any concentration of phosphate from M to M/30 was significantly different from the means at adjacent concentrations, and (b) that the value given by tissue from very young plants at any concentration of phosphate was significantly different from the value given by tissue from young plants at the same concentration.]

In the diagram are given progress curves for the oxygen uptake of individual samples of about equal weight, one from each experiment of the first series; it will be seen that the depressing action of the stronger solutions of phosphate is apparent throughout the period of observation. It will also be observed that the rate of oxygen uptake is very nearly constant in every case (i.e. the 'progress curves' approach very closely to straight lines), and is maintained at the same level right to the end of the six-hour period. This constant rate of oxygen uptake appears to be characteristic of the stem tissue, as it was observed in every sample throughout the experiments. Marked curling of

the stem slices was observed to take place in the stronger solutions of phosphate (M to M/10); but the slices never became flaccid in the six-hour period, and indeed retained their normal turgidity even after standing for twenty-four hours.



Very young plants. Oxygen uptake of individual samples of tissue in water and in various concentrations of acid potassium phosphate.

The first series of experiments was then repeated exactly with plants infected with aucuba mosaic virus, and in addition a few experiments were performed with tissue from old diseased plants (i.e. plants that had flowered).

It will be seen that the results agree with those obtained with healthy plants, both in the depression of oxygen uptake in the stronger concentrations of phosphate, and in the consistently lower level of respiration in tissue from plants in the 5th leaf stage, as compared with slightly older plants.

As might be expected, the tissue from old plants has a still lower level of respiration (cf. Kidd, West, and Briggs, 1921). But the low level of oxygen uptake in tissue from very young plants, observed with both healthy and diseased plants, is a more unexpected result.

That a low respiration rate is not an intrinsic property of young tissue is shown by a consideration of the results obtained when samples taken from the apex of the stem of a plant in the 6th to 12th leaf stage are compared with samples taken farther and farther down the stem. A plant in this stage is

TABLE III

Oxygen Uptake of Slices of Tomato Stem (Diseased Plants)

Concentration of KH_2PO_4 .	Mean oxygen uptake ($\mu\text{l./gm./hr.}$).		
	Very young plants.	Young plants.	Old plants.
M	13.9	—	14.3
M/3	27.2	55.7	35.0
M/10	57.7	102.7	54.3
M/30	78.0	121.1	47.8
M/100	*100.5	109.0	—
Water	*88.1	106.7	—

* (2 samples only.)

TABLE IV

Position Gradient of Respiration

Position on stem.	No. of samples.	Mean oxygen uptake ($\mu\text{l./gm./hr.}$).
Apex	5	163
2nd internode .	6	148
Middle part .	3	97
„	3	124
„	3	114
„	3	104
Near ground-level.	3	98

still actively growing, and the apex of the stem is composed of more recently formed, and therefore younger, tissue than the lower part of the stem.

The results of one such experiment are given in Table IV; a single well-grown healthy young plant was used, and the oxygen uptake of samples taken farther and farther down the stem measured in M/30 KH_2PO_4 .

In a similar experiment, with a virus plant of the same age, samples of tissue from the apex of the stem had a mean oxygen uptake of 169 $\mu\text{l./gm./hr.}$, while the main part of the stem gave mean values of 100–120 $\mu\text{l.}$ So in both cases the youngest tissue, taken from the apex of the stem, had a much higher respiration rate than the slightly older tissue from the lower part of the plant.

It is therefore unlikely that the low level of oxygen uptake in tissue from plants in the 5th leaf stage was caused by the youth *per se* of the tissue, and another cause must be looked for. Among the probable causes is a lack of available respiratory substrate; to obtain further information on this point the experiments reported below in section 2 of this paper were carried out.

2. *The effect of added carbohydrates.*

A series of experiments was carried out to test the effect of adding carbohydrate to tissue from plants of different ages. The carbohydrates used were the monosaccharides glucose and fructose; their effect on the oxygen

TABLE V
Addition of Glucose to Tissue from Old Plants

Type of plant.	Glucose per cent.	Mean oxygen uptake (μ l./gm./hr.).		Increase per cent.
		Control.	Treated.	
After flowering . . .	0.5	95	99	5 (not sig.)
Very old . . .	0.5	95	95	0
	0.5	48	56	15 (not sig.)

TABLE VI
Addition of Carbohydrate—Young Plants (Healthy)

Carbohydrate.	Concentration per cent.	Mean oxygen uptake (μ l./gm./hr.).	
		Control.	Treated.
{ Glucose . . .	0.2	*96	96
{ Fructose . . .	0.2	—	98
Glucose . . .	0.2	133	135
" . . .	1.0	143	153
Fructose . . .	1.0	163	161
Glucose . . .	0.5	103	105

(plant 132 hours in dark).

* (2 samples).

uptake of tomato tissue appeared to be identical (except in one anomalous experiment which we were unable to confirm).

[M/30 acid phosphate was used as the suspension medium, as it had already been found to give the maximum oxygen uptake; in each experiment a sugar solution of the required strength (0.2, 0.5, or 1.0 per cent.) was made up in sterile phosphate and added to three of the Barcroft bottles, while phosphate alone was added to the other three, thus providing a separate control for each experiment. (If glucose and fructose were used in the same experiment, they were added to two bottles each, leaving two control samples.)]

In the first set of experiments old plants were used; only a limited amount of material, and that all from virus plants, was available. The results, given in Table V, indicate that a negligible increase of oxygen uptake was obtained on the addition of glucose, although the uptake of the control samples was small.

When 'young' plants (in the 6th–12th leaf stage) were used, the level of oxygen uptake in the controls was rather higher; but in this case also there was not a significant increase in oxygen uptake on the addition of carbohydrate. Some typical results are given in Table VI, which includes two experiments with a 1 per cent. concentration of sugar, and one where the plant was kept in the dark before the experiment.

When tissue from very young plants (5th leaf stage) was used, on the other

hand, a very marked increase in oxygen uptake was produced in every case by the addition of carbohydrate. The results of some experiments with healthy 5th leaf-stage plants are given in Table VII. In the later experiments of this set the plant was kept in the dark for some time before the experiment;

TABLE VII
Very Young Healthy Plants—Addition of Carbohydrate

Hours in dark.	Carbohydrate.	Concentration per cent.	Oxygen uptake (μ l./gm./hr.).		Increase per cent.
			Control.	Treated.	
0	{ Glucose	0.2	*84	107	27
	{ Fructose	0.2	—	107	27
0	Glucose	0.2	60	105	75
0	Glucose	0.2	68	124	82
16	{ Glucose	0.2	*46	65	46
	{ Fructose	0.2	—	67	50
40	{ Glucose	0.5	*47	79	58
	{ Fructose	0.5	—	76	59
48	Glucose	0.5	46	88	91
64	{ Glucose	0.5	*37	84	127
	{ Fructose	0.5	—	67	80
72	Glucose	0.5	41	59	44

* (2 samples).

TABLE VIII
Very Young Diseased Plants—Addition of Glucose

Hours in dark.	Concentration per cent.	Oxygen uptake (μ l./gm./hr.).		Increase per cent.
		Control.	Treated.	
16	0.5	19	51	170
96	0.5	34	83	144
200	0.5	24	46	100

this treatment reduced the oxygen uptake of the controls, and augmented slightly the effect of added carbohydrate. The increase in oxygen uptake is expressed as a percentage of the control uptake; i.e. by the expression $\frac{(b-a)}{a} 100$, where a is the mean oxygen uptake of the control, and b of the treated, tissue.

Owing to a shortage of material only a few experiments could be performed on diseased 5th leaf-stage plants; the results are given in Table VIII.

These three experiments, as far as they go, show that the oxygen uptake of the control tissue is reduced to a greater extent in diseased than in healthy plants, by keeping the plant in the dark before the experiment, and that the addition of glucose produces a proportionately greater rise in oxygen uptake.

Respiratory quotient.

Some measurements of the respiratory quotient were performed on tissue from healthy young plants. The technique used was that described by Dixon

(1934), in which the measurements of oxygen uptake, carbon-dioxide output, and bound carbon dioxide are performed on three different samples; the estimate of the respiratory quotient is therefore only approximate.

TABLE IX
Respiratory Quotient

Expt. no.	Oxygen uptake (μ l./gm./hr.).	R.Q. (CO_2/O_2).
1	137	0.94
2	184	1.02
3	158	1.03
4	158	1.02
5	144	1.01

None of the figures obtained for the respiratory quotient was significantly different from unity.

DISCUSSION

The method employed in this work appears to give, with a very small amount of material, an adequate measurement of the respiration of plant tissue. That the measurements of oxygen uptake recorded were in fact due to normal aerobic respiration is indicated by the following observations: (a) the slices of stem tissue were invariably found to take up oxygen at a constant rate which was maintained over the whole period of an experiment; (b) the estimates obtained of the respiratory quotient approached closely to unity; (c) the tissue slices used showed no sign of flaccidity after standing for twenty-four hours.

The effect of phosphate.

As the oxygen uptake of slices suspended in distilled water was only slightly less than that of slices suspended in M/30 phosphate, it is evident that there was no question of phosphate starvation in the tissue used. M/30 phosphate was probably effective in producing the largest oxygen uptake observed because it approached most nearly, in acidity and tonicity, to the cell-sap of all the solutions used. It certainly caused less curling of the tissue slices than the other solutions. The marked depression of respiration observed in the presence of M/10 or stronger concentrations of phosphate is probably due to their being hypertonic to the cell-sap; it is interesting to compare with our results those of van Heyningen (1935) on the oxygen uptake of a variety of animal tissues. Van Heyningen criticizes the work of Dixon and Elliott (1929) on the ground that they used a buffer solution containing M/10 phosphate in their experiments, and that this concentration was too strong; the medium that he used contained M/40 phosphate, and in this medium he obtained consistently higher values of oxygen uptake than those recorded by Dixon and Elliott for the same animal material.

The effect of age on oxygen uptake.

In any study on the effect of age on the respiration of isolated parts of a plant it is important to distinguish between the age of the plant from which the part has been removed and the stage of development reached by that particular part. That a failure to make this distinction may lead to misconceptions of the effect of age on physiological processes has been shown by Richards, who points out (1934) the fallacies underlying the method of estimating the effect of age on, e.g. respiration, by performing measurements on successive leaves taken from the same plant, and states that corresponding leaves from plants of different ages must be used in such an estimation, for, unless this is done 'it is impossible to separate effects which may be possibly ascribed to age as such from those due to change in conditions of nutrition, etc.' This point is emphasized in the work reported in this paper; for instance, the extreme top of a plant in the 6th to 12th leaf stage may be regarded as young tissue in one sense, as it is the last formed part of the plant; but we have shown that it has a very much higher rate of respiration than tissue removed from a very young plant. The tissues at the top of a mature plant are not physiologically equivalent to those of a very young plant.

In a well-grown young plant there must be an ample supply, throughout the plant, of respirable material formed by photosynthesis, and under these circumstances very young tissue exhibits a higher rate of oxygen uptake than older tissue. It is therefore probable that the low rate of oxygen uptake exhibited by tissue from very young plants is caused by a lack of available substrate, most of the respirable material having been used up in tissue formation, and not by a lack of activity in the respiratory enzyme system.

This suggestion is confirmed by the results obtained on the addition of sugar to the respiratory tissue. In young plants, where both an efficient respiratory system and an ample supply of respiratory substrate are present, the oxygen uptake of untreated tissue is high and is not raised by the addition of carbohydrate. In both very young and very old plants the oxygen uptake of untreated tissue is low, but from different causes. In old plants the uptake is only very slightly raised by added carbohydrate, and the low respiration rate is presumably due to a decline in the activity of the respiratory system. In very young plants the respiratory system is highly active, but there is a lack of available substrate, and accordingly the addition of glucose or fructose produces a very marked rise in oxygen uptake.

The results obtained with added carbohydrate also indicate the one point of difference between healthy- and virus-diseased tissue that was observed in these experiments. It appears that respiratory substrate is lost to a greater extent from virus plants when they are kept in the dark than from healthy plants. Glucose, added to the tissue from starved virus plants, produced a greater proportional increase of respiration than in the corresponding normal tissue. The incompleteness of these results prevents any very definite conclusion being drawn from them, but they partly confirm a suggestion made

by Caldwell (1934) that the enzymes concerned in the preliminary stages of respiration were more active in virus-diseased than in healthy plants. The preliminary experiments, with extracted and with purified juice from virus plants, gave no indication that the virus agent itself had any oxygen uptake. This confirmed the view that the increase in the respiration rate of the tissues of diseased plants was not associated with the respiration of the virus agent itself.

SUMMARY

1. The oxygen uptake of thin slices of tomato stem tissue was measured in Barcroft respirometers, and found to be maintained at a constant rate over a six-hour period.

2. The highest values for oxygen uptake were observed in presence of M/30 potassium dihydrogen phosphate; measurements in distilled water gave slightly lower values, and stronger solutions of phosphate produced a marked depression of oxygen uptake.

3. Tissue from very young plants, in the 5th leaf stage, showed a lower level of oxygen uptake than tissue from slightly older plants, up to the 12th leaf stage. A low level of oxygen uptake was also observed in tissue from old plants that had flowered.

4. The small oxygen uptake of tissue from very young plants was markedly raised by the addition of glucose or fructose, but no such rise was observed on adding sugar to tissue from very old plants.

5. It is concluded that the oxygen uptake is limited in old plants by the activity of the respiratory enzyme system, and in very young plants by the amount of available respiratory substrate.

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Observations on the Oxygen Uptake of Isolated Plant Tissue

II. The Effect of Inhibitors

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With three Figures in the Text.

INTRODUCTION

DURING an attempt to study the possible effects of aucuba mosaic virus upon the respiratory activity of infected plants a number of observations were made on the oxygen uptake of excised tissue from the stem of the tomato (*Lycopersicum esculentum*). Some of these observations have been reported in a previous paper (Caldwell and Meiklejohn, 1937), which deals with the effect of phosphate and of added carbohydrate. In the present paper are described a further series of experiments, in which substances known or thought to inhibit enzyme action were added to the tomato tissue.

METHODS

The methods and material used have been described in detail in the earlier paper. The stem tissue was cut into slices 0.5 mm. thick, which were suspended in 3 ml. of M/30 acid potassium phosphate (KH_2PO_4), and their oxygen uptake at 26° C. was measured by means of Barcroft differential manometers over a six-hour period. The method, previously described, of providing a separate control for each experiment, was followed throughout; the inhibitor was added to three of each set of six Barcroft bottles after weighing, and just before they were attached to the manometers. These bottles contained either 2.9 or 2.7 ml. of phosphate solution, and either 0.1 or 0.3 ml. of a solution of the poison of appropriate strength was added to bring the volume up to 3 ml. (e.g. to obtain a concentration of M/300 KCN in the bottles, 0.1 ml. of an M/10 solution of potassium cyanide was added to 2.9 ml. of phosphate solution). The amount of poison added is expressed throughout in terms of the concentration in the bottles at the beginning of the experiment.

The amount of oxygen taken up is expressed as $\mu\text{l.}$ of gas (dry at N.T.P.) per hour per gramme fresh weight of tissue, and each figure given in the tables is the mean of three parallel samples. The degree of inhibition of oxygen uptake, where this occurred, is expressed as a percentage of the uptake of the control, i.e. by the expression $\frac{(a-b)}{a} \times 100$, where a is the mean oxygen uptake of the control, and b of

the treated material. The reality of the observed differences between the control and the treated material was tested by calculating the standard errors of these mean values (Fisher, 1934). As was stated in the previous paper, the variation of the individual readings with this material is such that differences of the order of 10 per cent. of the control cannot be detected. In a few cases small differences between the mean values for control and treated material have been found not to be significant, and are marked accordingly in the tables. The differences not so marked have been tested and found to be highly significant.

RESULTS

(a) Cyanide.

The addition of potassium cyanide to the phosphate solution in which the stem slices were placed produced a very marked inhibition of oxygen uptake. As will be seen by reference to Table I, an inhibition amounting to 85 per

TABLE I
Effect of Cyanide on the Oxygen Uptake of Tomato Tissue

Concentration of cyanide.	Solution in.	Type of plant.	Oxygen uptake ($\mu\text{l./gm./hr.}$).		Inhibition per cent.
			Control.	Treated.	
M/30	M/30 KH_2PO_4	Healthy young	127	19	85
M/300	"	"	97	21	79
"	"	"	124	20	84
"	"	Virus young	77	15	80
"	Water	Healthy young	98	14	85
"	M/30 K_2HPO_4	"	85	12	85
M/3,000	M/30 KH_2PO_4	"	114	46	61
M/30,000	"	"	104	97	5 (not sig.)
"	M/30 K_2HPO_4	"	76	85	0
"	"	"	74	72	0

cent. of the uptake of untreated material was produced by M/300 cyanide; on raising the concentration of cyanide to M/30 no further inhibition was produced, so that the cyanide-stable part of the respiratory system accounts for about 15 per cent. of the total oxygen uptake. These figures are very similar to those obtained by van Heyningen (1935) with animal tissue, both in the amount of cyanide required for maximal inhibition, and in the degree of inhibition (80–90 per cent. for most animal organs) produced.

It will be seen that the inhibition produced by M/300 cyanide is of the same order with virus material as with healthy, and is the same in water or alkaline phosphate as in the acid phosphate solution used as a standard. The disadvantage of using an acid solution for these experiments, however, is that there is a loss of cyanide from the solution during the experiment, which

affects the results with low concentrations of cyanide. (A method for avoiding this loss has been described by Krebs (1935), but it was not employed in our experiments.) This point is brought out in Fig. 1, which represents measurements of total oxygen uptake during the course of an experiment, from single samples of tissue of approximately equal weights. It will be noticed that in presence of $M/30,000$ cyanide, the oxygen uptake increased during the experiment; the effect of the cyanide wore off, as more and more of it was lost from the solution. In the case of $M/30,000$ cyanide there was an initial inhibition only; but with $M/300$ or $M/30$ there was so much cyanide present that the loss by evaporation was not noticeable.

This diagram also illustrates the fact, to which reference has previously been made, that the oxygen uptake of the control tissue was constant throughout the period of the experiment (i.e. successive readings give a straight line when plotted as a graph); this has been found to hold for untreated material throughout these experiments.

The action of cyanide in lowering the oxygen uptake is at least partly reversible, i.e. the effect disappears on the removal of the cyanide, as is shown by an experiment carried out with tobacco stem tissue (no tomato tissue being available). In this experiment the average oxygen uptake of three control samples (in $M/30$ KH_2PO_4) was $76 \mu\text{l./gm./hr.}$ The three treated samples were allowed to respire for one hour in an $M/300$ solution of cyanide in $M/30$ phosphate, when their average oxygen uptake was $8 \mu\text{l./gm./hr.}$, giving an inhibition of 89 per cent. The treated samples were then removed, washed in two changes of sterile phosphate solution, and placed in fresh bottles with 3 ml. of phosphate solution in each. Their average oxygen uptake, measured

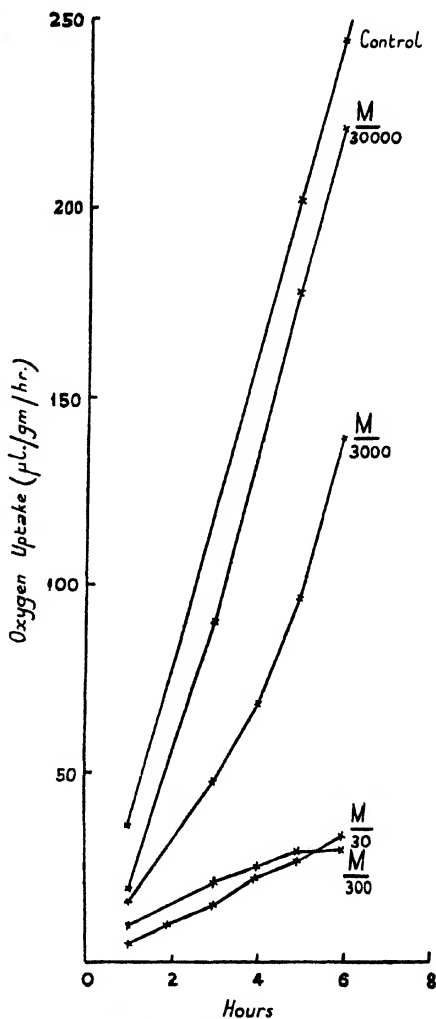


FIG. 1. Oxygen uptake of single samples of tissue in the presence of KCN.

over four and a half hours, was then 33 $\mu\text{l./gm./hr.}$, or four times the uptake in presence of cyanide, a recovery of nearly half the original rate. Total recovery was not observed, probably because the washing process removed some of the soluble carbohydrate in addition to the cyanide, and because the washing process was not absolutely complete.

As very weak concentrations of cyanide have been shown to have a stimulating effect on the respiration of some plant tissues (Hanes and Barker, 1930), and

TABLE II

Respiratory Quotients in Presence and Absence of Cyanide

Type of plant.	Treatment.	Oxygen uptake ($\mu\text{l./gm./hr.}$).	R.Q. (CO_2/O_2).
Healthy young	+ M/30,000 KCN	114	1.02
"	Control	158	1.02
"	+ M/30,000 KCN	139	1.02
"	Control	144	1.01

as we had observed no stimulation of oxygen uptake due to cyanide in our experiments, it was thought desirable to measure the respiratory quotient of tomato tissue in the presence of very weak cyanide, to ascertain whether there was any stimulation of carbon-dioxide output. The method described by Dixon (1934) was used, in which the oxygen uptake is measured in one apparatus and the carbon-dioxide output measured in another, while a third is used for the measurement of retained carbon dioxide. As Dixon pointed out, this direct method is not adapted to accurate measurement of the respiratory quotient, as the oxygen and carbon-dioxide estimations have to be performed on different samples. The values obtained for the respiratory quotient (given in Table II) must, therefore, be regarded as approximate only, more especially as the figures given for oxygen uptake are taken from single samples, no duplicates being possible.

These results, though approximate, show no perceptible alteration of the respiratory quotient in presence of weak cyanide; if any stimulation in carbon-dioxide output took place under such conditions, it must have been exceedingly small.

Two experiments were performed to test the effect of cyanide on oxygen uptake in the presence of added glucose; for this purpose tissue from very young plants was employed, as it was known to respond to the addition of glucose (Caldwell and Meiklejohn, 1937).

In both cases, although the respiration in absence of cyanide was raised on the addition of glucose, the residual respiration in presence of cyanide was the same with or without glucose. These results agree with those obtained by Genevois (1929).

(b) *Sodium fluoride.*

Sodium fluoride produced a marked inhibition of the oxygen uptake, but only in relatively high concentrations, the maximal effect being observed in

the presence of M/30 fluoride; the effect in water was the same as in phosphate solution. This inhibition is not reversible; tissue subjected to treatment with M/300 fluoride for an hour showed no recovery of oxygen uptake after washing. The results are given in Table IV.

TABLE III
Effect of Cyanide in Presence of Glucose (Very Young Plants)

Type of plant.	Treatment (in M/30 KH_2PO_4).	Oxygen uptake ($\mu\text{l./gm./hr.}$).		Change in uptake (per cent. of control).
		No KCN.	KCN present.	
Healthy	Control	41		
	+M/75 KCN		13	-68
	0.5 per cent. Glucose	59		+44
	+M/75 KCN		11	-80
Virus	Control	34		
	+M/150 KCN		15	-56
	0.5 per cent. Glucose	83		+144
	+M/75 KCN		14	-82

TABLE IV
Effect of Sodium Fluoride on the Oxygen Uptake (Healthy Young Plants)

Concentration of NaF.	Solution in.	Oxygen uptake ($\mu\text{l./gm./hr.}$).		Inhibition per cent.
		Control.	Treated.	
M/30	M/30 KH_2PO_4	99	8	92
"	Water	122	11	91
M/300	M/30 KH_2PO_4	130	32	75
M/3,000	"	101	92	9
M/300	"	106	44	
			(before washing)	
	(no recovery)		37	
			(after washing)	

The course of respiration of individual samples of tissue in presence of different concentrations of fluoride is shown in Fig. 2. There was no diminution in effect due to loss of inhibitor during the experiment in this case; on the contrary, an increase in inhibition as the experiment proceeds may be seen in the curve for M/300 fluoride.

(c) *Iodoacetic acid.*

Iodoacetic acid strongly inhibited the oxygen uptake of tomato tissue at very weak concentrations.

The inhibition caused by iodoacetic acid is not constant throughout the period of observation; in Fig. 3 are given the actual readings from control and treated tissue samples of approximately equal weight, which show that the inhibition increases with time. This is particularly noticeable in the curve for M/10,000 iodoacetic acid. Apparently this particular inhibitor penetrates into the tissue far more slowly than, for instance, cyanide. This inhibition

is not reversible; the results obtained by allowing the tissue to respire for one hour in presence of iodoacetate, washing it with sterile phosphate, and measuring the oxygen uptake in fresh phosphate, are given in Table VI.

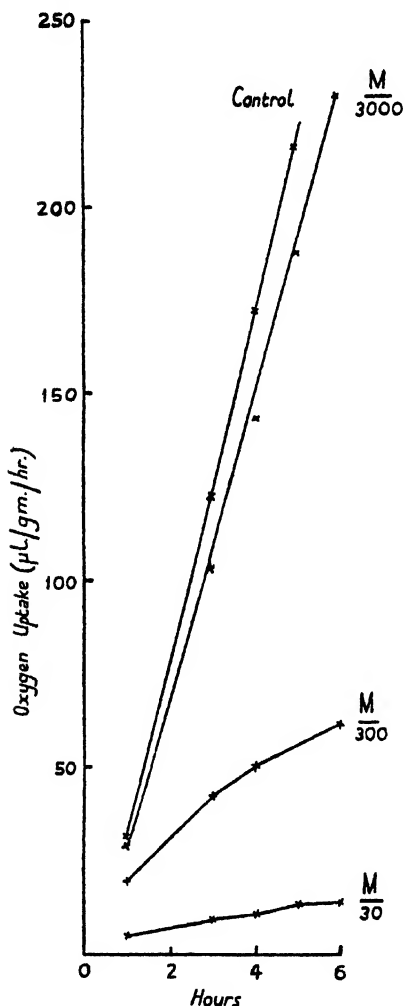


FIG. 2. Oxygen uptake of single samples of tissue in the presence of sodium fluoride.

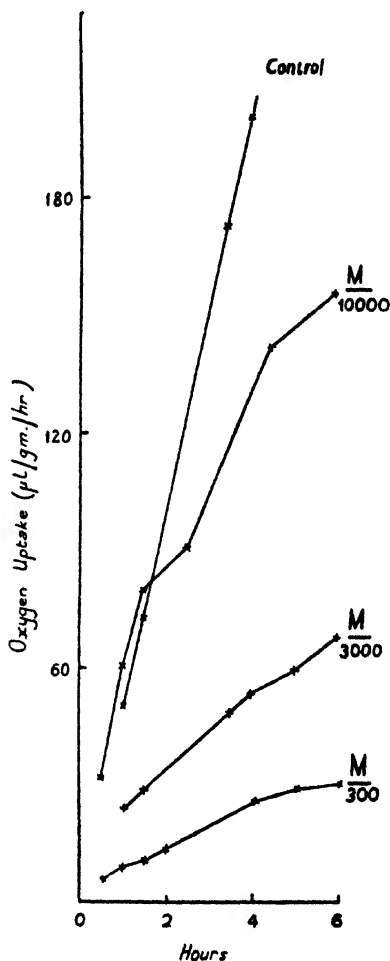


FIG. 3. Oxygen uptake of single samples of tissue in the presence of iodoacetic acid.

(The first experiment was done with tobacco stem tissue, owing to a shortage of tomato plants.) No recovery was observed after washing, even at $M/10,000$ iodoacetate; in fact, washing appears to reduce the oxygen uptake still further. This may be because the inhibition increases with time.

As, in some animal tissues, the oxygen uptake in presence of iodoacetate can

be restored to its original value by the addition of lactate (Needham, 1932), it was thought of interest to examine the effect of iodoacetic acid on tomato stem tissue in presence of lactic acid. Two preliminary experiments with

TABLE V

The Effect of Iodoacetic Acid on the Oxygen Uptake of Tomato Tissue

Concentration of iodoacetate.	Type of plant.	Oxygen uptake (μ l./gm./hr.).		Inhibition per cent.
		Control.	Treated.	
M/300	Old healthy	83	8	91
M/3,000	"	54	12	77
"	Young healthy	132	30	77
"	"	109	33	70
M/10,000	"	141	51	64
M/30,000	Old healthy	46	31	32
M/300	Young virus	103	2	98
"	"	140	8	94
M/3,000	"	109	19	82
M/30,000	Old virus	60	53	(12) (not sig.)

TABLE VI

Effect of Washing Tissue treated with Iodoacetate (Healthy Plants)

Concentration of iodoacetic acid.	Type of plant.	Oxygen uptake (μ l./gm./hr.).		
		Control.	Treated. (before washing).	Treated. (after washing).
M/300	Old tobacco	92	30	11
M/3,000	Young tomato	122	57	24
"	"	141	26	18
M/10,000	"	141	98	72

TABLE VII

Lactic Acid and Iodoacetic Acid (Young Healthy Plants)

Concentration of iodoacetic acid.	pH.	Concentration of lactic acid.	Oxygen uptake (μ l./gm./hr.).	
			Without lactic acid.	With lactic acid.
0	4.6	0.1 per cent.	67	77
0	4.6	0.5 "	40	10
M/3,000	3.0	0.5 "	38	0
"	4.8	0.5 "	28	17
"	7.2	0.5 "	*56	*60
0	7.2	0.5 "	*73	

* 2 samples.

lactate alone in acid phosphate solution showed that it was only utilized to a limited extent as a respiratory substrate (first two lines of Table VII). When lactic acid was added to the phosphate solution (M/30 KH_2PO_4) without neutralization, the resulting solution had a pH value of 3.0, and this, in conjunction with iodoacetic acid, completely killed the tissue, making it flaccid, and stopping all uptake of oxygen. Another experiment was therefore performed in which the lactic acid in the acid phosphate solution was neutralized

to pH 4.8; and a third experiment was performed in a neutral buffer solution (equal parts of M/30 KH_2PO_4 and M/30 K_2HPO_4 , pH 7.2).

In acid solution the lactic acid depressed still further the lowered oxygen uptake produced by iodoacetic acid; even in neutral solution, iodoacetate and lactate together do not give so great an uptake as that recorded in the absence of both.

(d) *Sodium azide.*

Sodium azide (NaN_3) is known to have a pronounced inhibiting action on catalase (Keilin and Hartree, 1934). In these experiments it was found to inhibit oxygen uptake to a marked degree in the standard acid phosphate

TABLE VIII

Effect of Sodium Azide on the Oxygen Uptake of Healthy Tissue

Concentration of azide.	Solution in.	Type of plant.	Oxygen uptake ($\mu\text{l./gm./hr.}$).		Inhibition, per cent.
			Control.	Treated.	
M/300	Acid phosphate	Old tomato	70	4	94
M/3,000	"	Old tobacco	85	6	92
"	"	Young tomato	170	24	86
M/30,000	"	Old tobacco	85	49	42
M/300,000	"	"	84	81	0
M/300	Alkaline phosphate	Young tomato	144	55	61
M/30,000	"	"	125	114	(9) (not sig.)

solution; under these conditions the maximal inhibition was attained already at a concentration of M/3,000, and M/30,000 azide gave a considerable inhibition. In alkaline solution (M/30 K_2HPO_4), on the other hand, the effect was much reduced, M/300 azide giving 60 per cent. inhibition only instead of over 90 per cent. as in acid solution. The results are given in Table VIII; as before, some of the experiments were performed on tobacco plants.

The action of sodium azide was reversible; tobacco stem tissue treated with M/3,000 sodium azide for an hour, then washed and allowed to respire in fresh phosphate, showed a recovery of 35 per cent. When M/300 azide was used, no recovery on washing was observed; but as very dilute azide gives a considerable inhibition, M/300 is probably too strong a solution to be adequately removed by washing.

(e) *Malachite green.*

Malachite green is typical of the basic dyestuffs, which are known to be powerful inhibitors of the dehydrogenases (Quastel and Wheatley, 1931). When applied to tobacco stem tissue it was found to have a powerful inhibiting action on the oxygen uptake at extraordinarily low concentrations. It was the only substance used which gave a considerable inhibition at a concentration as low as 1/300,000 (about M/100,000).

(f) *Narcotics.*

The narcotics urethane and phenyl urethane are known to inhibit dehydrogenase systems, but only in relatively strong concentrations (Warburg, 1930; Sen, 1931). With tobacco tissue quite strong solutions gave only partial

TABLE IX

Effect of Washing Tissue treated with Sodium Azide (Healthy Plants)

Concentration of azide (in M/30 KH_2PO_4).	Type of plant.	Oxygen uptake ($\mu\text{l./gm./hr.}$).		
		Control.	Treated (unwashed).	Treated (after washing).
M/300	Old tomato	33	0	4
"	Young tobacco	130	3	4
M/3,000	Old tobacco	93	10	33

TABLE X

Effect of Malachite Green on the Oxygen Uptake of Tobacco Tissue

Concentration of malachite green.	Type of plant.	Oxygen uptake ($\mu\text{l./gm./hr.}$).		Inhibition per cent.
		Control.	Treated.	
1/3,000	Young healthy	109	23	79
1/30,000	"	104	39	61
1/300,000	"	114	54	44
1/3,000,000	"	111	105	(5) (not sig.)

TABLE XI

Effect of Urethanes on the Oxygen Uptake of Tobacco Tissue (Healthy)

Substance.	Concentration.	Oxygen uptake ($\mu\text{l./gm./hr.}$).		Inhibition per cent.
		Control.	Treated.	
Urethane	M/3	85	30	64
"	M/30	79	71	(10) (not sig.)
Phenyl } Urethane }	saturated	93	22	76
	M/300	110	75	31

inhibition of oxygen uptake; in fact with phenyl urethane the insolubility of the substance made it difficult to get a solution strong enough to show any pronounced action, and a saturated solution with some undissolved solid still present was added to the stem slices.

(g) *Amyl alcohol.*

The vapour of amyl alcohol has been found to have a stimulating effect on the carbon-dioxide output of apples (Caldwell, 1931). When added to the solution in the Barcroft manometer vessel, a strong solution of iso-amyl alcohol (1/30) had a very strongly inhibiting effect on oxygen uptake; the control samples of tomato stem tissue had an average oxygen uptake of 126 $\mu\text{l./gm./hr.}$; in presence of 1/30 iso-amyl alcohol this was reduced to 5 $\mu\text{l./gm./hr.}$, an inhibition of 96 per cent. A much weaker solution (1/3,000) had no effect, neither stimulating nor inhibiting; the average oxygen uptake of both control and treated material was 97 $\mu\text{l./gm./hr.}$

DISCUSSION

The substances applied to the tomato tissue in these experiments were all known to be poisons with an inhibiting effect on one or more enzyme systems. They were all found to affect the oxygen uptake of the tissue in substantially the same way; they gave an inhibition increasing with their concentration (in some cases to a limiting value, e.g. cyanide), and if the concentration was reduced below the point where inhibition occurred, no effect was produced. Now it is well known (see e.g. Irving, 1911, Hanes and Barker, 1930) that poisons applied to green plant material as vapour in the air respired have an inhibiting effect upon respiration at high concentrations, but a stimulating effect in very small doses. With the very different technique employed in our experiments, where the poisons were applied in solution to cut material, no stimulation of oxygen uptake was observed to result from the application of smaller doses than those which produced inhibition. This point is further illustrated by the case of amyl alcohol, which stimulates the carbon-dioxide output of apples when applied as vapour (Caldwell, 1931), but in solution was found to have no effect on oxygen uptake at a low concentration and to inhibit it almost completely at a high concentration.

The effects observed on the addition of individual poisons to tomato stem slices show a very close resemblance, both in kind and in degree, to results obtained by other workers with animal tissues and bacteria.

The results which we have obtained with cyanide are very strikingly similar to those recorded by Dixon and Elliot (1929) and van Heyningen (1935) on the action of cyanide on the oxygen uptake of slices of various animal tissues. As in animal tissue slices, about 85 per cent. of the total oxygen uptake is accounted for in plant tissue slices by a system (the 'atmungsferment' of Warburg) which is reversibly poisoned by cyanide. The results obtained on the addition of cyanide in presence of added glucose agree with those of Genevois (1929), and might be regarded as confirming the conclusion he draws from them—that the oxidation of carbohydrate is effected only by that part of the respiratory system which is sensitive to cyanide. It is possible, however, that the small fraction of the respiratory enzyme system which is resistant to cyanide is also concerned with the oxidation of glucose, but is saturated by a very minute quantity which would presumably be present even in conditions of extreme starvation, and the addition of more glucose would have no effect on the oxygen uptake.

The analogy between the respiratory systems of animal and plant tissue must not be pushed too far; the results obtained on the addition of fluoride and iodoacetic acid to tomato tissue illustrate this point. Both these reagents inhibit to a marked degree the oxygen uptake of plant as well as of animal tissue. But in animal tissue they produce their effect by interrupting a process—the conversion of glucose to lactic acid—which probably does not take place in plant tissue (see Genevois, 1929). This conclusion is supported by the observation that the inhibition of oxygen uptake produced in tomato

tissue by iodoacetate was not removed by the addition of lactate, which removes the inhibition observed with chicken embryos in presence of iodoacetic acid (Needham, 1932). It is of interest to note that the slowness of penetration into tissues exhibited by iodoacetic acid in Needham's experiments was observed by us at certain concentrations, see the curve for M/10,000 iodoacetic acid in Fig. 3.

The marked inhibition of oxygen uptake in tomato tissue produced by sodium azide, which has been shown by Keilin and Hartree (1934 and 1936) to act as a specific inhibitor of catalase, may indicate that catalase is an essential part of the respiratory system in plants (see Stiles and Leach, 1932). The fact that azide has a more powerful inhibiting action in acid solutions (Keilin and Hartree, 1936) has also been observed in our experiments.

The results obtained with malachite green and with urethanes are very similar to those formerly recorded (Quastel and Wheatley, 1931; Sen, 1931) with animal tissues and with bacteria.

Taken together, the effects of poisons recorded in this paper show the very great similarity that must exist between the cellular respiration of green plants and that of animals, and also of colourless plants such as yeast.

SUMMARY

Substances known to inhibit enzyme action were added to slices of tomato stem tissue, and their effect on the oxygen uptake of the tissue was measured. All the substances showed an inhibiting action which increased with their concentration. Concentrations lower than those which inhibited oxygen uptake were found to have no stimulating effect.

Cyanide (M/300) produced a reversible inhibition of about 85 per cent. of the total oxygen uptake; no greater inhibition was produced by M/30 cyanide than by M/300.

Sodium fluoride and iodoacetic acid had an irreversible inhibiting action, and sodium azide a reversible one stronger in acid than in alkaline solution.

Malachite green was effective in very small doses, but the urethanes only in high ones.

Amyl alcohol was ineffective at 1/3,000, but produced almost complete inhibition at 1/30.

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AN INVESTIGATION OF NITROGEN UPTAKE IN MIXED CROPS NOT RECEIVING NITROGENOUS MANURE

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(With Plate VII)

INVESTIGATIONS performed during the last ten years (mainly at Valios Laboratorium, Finland, and at Rothamsted Experimental Station) have established that benefits are in some cases derivable by a non-legume growing alongside an inoculated legume in sand culture. These benefits are expressed by a gain of nitrogen and better growth of the non-legume, compared with the same species of non-legume grown without nitrogen either in single culture or in the presence of an uninoculated leguminous species. The effect of an inoculated companion legume on a non-legume may thus be similar to that obtainable by the application of nitrogenous manure to a non-legume in single culture; it appears that the leguminous component of a mixed crop can sometimes confer part of its nitrogen on the non-legume. This effect has not been demonstrated in the field, but certain Rothamsted experiments⁽⁵⁾ have shown that there was no significant gain in total nitrogen in a mixed crop when nitrogenous manures were applied.

Lipman⁽²⁾ suggested as long ago as 1910 that legumes may vary in their ability to act as donors of nitrogen to companion non-legumes. The converse suggestion, that non-legumes may vary in their ability to utilize the nitrogen made available by companion leguminous plants, has been made by Nicol⁽³⁾. Nilsson-Leissner⁽⁴⁾ has shown that there are both varietal and specific differences in the ability of some grasses to utilize inorganic nitrogenous manures, and that these differences themselves vary according as to whether the grass is grown in presence or absence of clovers in the field.

The author has had the idea that just as legumes may vary in the ability to act as "donors" of their symbiotically fixed nitrogen, so some non-legumes may be better "acceptors" than others of such symbiotically

fixed nitrogen as is not taken up by the leguminous plants. The following paper contains two sets of experimental results obtained in an attempt to examine the influence of four species of nodule-bearing leguminous plants upon English rye-grass and barley in sand culture. The first experiment was performed at Puławy in 1935, the second at Rothamsted in 1936.

I. EXPERIMENT AT PUŁAWY IN 1935

The experiment was carried out in pots containing 30 kg. of drift sand, in an unheated greenhouse. The sand had been washed four times in tap water, and twice in distilled water, before potting. The pots were divided into five series, each containing five replicates. The following nutrient salts were added in one dose to each pot before sowing:

K_2HPO_4	4.4 g. per pot
KH_2PO_4	0.3 ,,
$MgSO_4 \cdot 7H_2O$	0.3 ,,
KCl	0.3 ,,
$FeCl_3$ anhydride	0.075 ,,

The *pH* of the sand during the vegetation season was about 6.5. The seeds of the leguminous plants were inoculated with an effective strain of the specific nodule bacteria. The pots were watered with distilled water to a constant weight (60 per cent of the full water capacity of sand).

The scheme of the experiment is given in Table I.

Table I

Series	Plants
A	20 plants of English rye-grass
B	10 plants of English rye-grass
BC	10 plants of serradella and 10 plants of rye-grass
BD	10 plants of red clover and 10 plants of rye-grass
BE	10 plants of peas and 10 plants of rye-grass

Sowing was done on 1 July, and after 13 weeks' growth all plants were harvested on 30 September. At that time, serradella and clover were still in flower with some pods formed, and the peas were nearly ripe. The pots were left unwatered for several days before harvest, till the sand became dry, the plants were then lifted out of the sand, gently shaken and washed free from sand; the bulk of the sand was passed through a sieve to remove fragments of root.

Length of roots, air-dry weight and nitrogen content of plants and nitrogen content of sand were ascertained (unfortunately the sand was

not analysed before use), the results being summarized in Tables II and III.

Table II. *Average air-dry weight of rye-grass, g. per pot*

Series	Total plant		Shoot		Root g.	Overall length of roots cm.
	g.	as % of B	g.	as % of B		
A. Rye-grass (20 plants)	1.25 ± 0.01	—	0.61 ± 0.06	—	0.64	20
B. Rye-grass (10 plants)	1.02 ± 0.12	100	0.54 ± 0.06	100	0.48	19
BC. Rye-grass (10 plants) + serradella	1.92 ± 0.27	188	1.08 ± 0.15	200	0.84	22
BD. Rye-grass (10 plants) + clover	2.14 ± 0.12	220	1.17 ± 0.06	217	0.97	29
BE. Rye-grass (10 plants) + peas	3.29 ± 0.35	322	1.72 ± 0.16	331	1.57	24

Table III. *Total and percentage nitrogen in dry matter of rye-grass per pot*

Series	Nitrogen %	Nitrogen mg.	Nitrogen as percentage of nitrogen in B
B. Rye-grass (10 plants)	0.78	7.96	100
BC. Rye-grass (10 plants) + serradella	0.92	17.66	222
BD. Rye-grass (10 plants) + clover	1.05	22.47	282
BE. Rye-grass (10 plants) + peas	1.17	38.49	483

It is evident that the leguminous plants grown with rye-grass had a significant effect upon the total yield and nitrogen yield in rye-grass. The best companion of rye-grass seems to be the pea (Pl. VII, fig. 1). In this series of pots (BE) the yield of rye-grass was three times, and the nitrogen yield about five times, as large as in the control pots. There was a little interpenetration of roots(1) in this series of pots. All roots were long and well developed.

In the clover series there was extensive interpenetration of roots, so that separation of the roots of the two species became very difficult. The effect of clover upon the rye-grass growth and nitrogen yield (as well as nitrogen content) was less marked than in the previously described series of pots.

In the serradella group the roots of rye-grass were badly developed and short, the tops were light green, but even so the yields of grass and of total nitrogen in the grass were twice as great as in the pots with rye-grass only.

The sand of the pea-rye-grass pots contained the most nitrogen after harvest (Table IV).

Table V represents the yield of leguminous plants per pot, and percentages and yield of total nitrogen.

Table IV. *Nitrogen in dry sand after harvest*

Series	%	g. per pot (29 kg. dry sand)
B. Rye-grass	0	0
BC. Rye-grass + serradella	0.0015	0.44
BD. Rye-grass + clover	0.0011	0.32
BE. Rye-grass + peas	0.0042	1.22

Table V. *Leguminous plants: yield and nitrogen content as percentage of dry matter*

Plants	Dry matter g. per pot	% nitrogen	Nitrogen g. per pot
Serradella	8.25	3.07	0.24
Red clover	23.70	3.20	0.75
Peas	77.31	2.66	1.97

Summarizing the nitrogen yield in (1) leguminous plants, (2) rye-grass, and (3) sand (per pot), it is evident that ten plants of peas have given the highest amount of assimilated nitrogen and ten serradella plants the smallest amount. On the other hand, per 100 g. of plants, the peas and red clover fixed a smaller total amount of nitrogen, as is shown in Table VI.

Table VI. *Total symbiotically fixed nitrogen during 13 weeks' growth*

Series	Nitrogen fixed by 10 plants	Nitrogen per 100 g. of legu- minous plant (dry matter)
	mg.	mg.
Serradella	702	8500
Red clover	1102	4640
Peas	3218	4160

II. EXPERIMENT AT ROTHAMSTED IN 1936

The experiment was performed in glazed earthenware pots, each holding about 20 kg. of sand, containing 0.0026 per cent of total nitrogen. 10 g. of precipitated chalk was well mixed with the sand before filling each pot, and 500 c.c. of the following nutrient solution was also added:

K_2SO_4	1.75	g.
K_2HPO_4	0.75	"
KH_2PO_4	0.75	"
$FeCl_3$ anhydride	0.075	"
$MgSO_4 \cdot 7H_2O$	0.75	"
$CaSO_4 \cdot 2H_2O$	0.6	"
NaCl	0.75	"



Fig. 1. Rye-grass grown in sand, alone or in association with leguminous plants. No nitrogen given as manure. 0 alone; 1, with serradella; 2, with clover; 3, with peas.

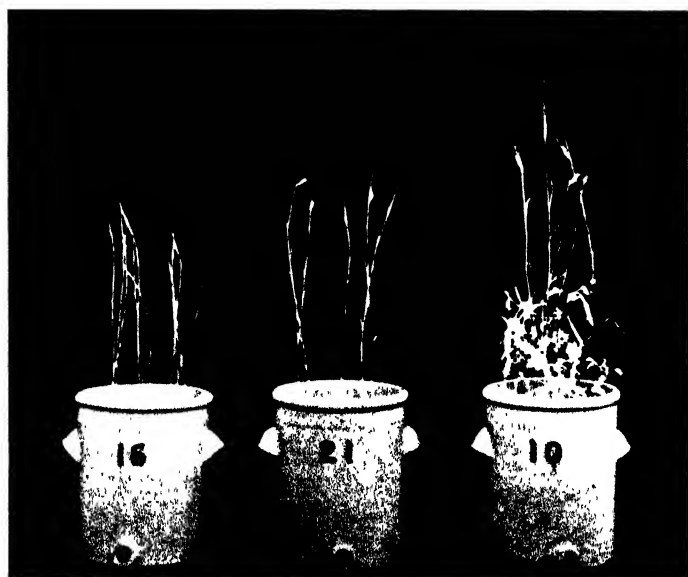


Fig. 2. Plants grown in sand without nitrogenous manure. Pot 16, ten barley plants; pot 21, five barley plants; pot 10, five barley plants + five peas.

The pots were weighed at the commencement of the experiment, and afterwards watered with rain water as required to bring them back to the initial weight. The experiment was divided into five series, each containing five replicates, as shown in Table VII.

Table VII

Series	Plants
A	10 plants of barley
B	5 plants of barley
BC	5 plants of barley and 5 plants of peas
BD	5 plants of barley and 5 plants of lucerne
BE	5 plants of barley and 5 plants of red clover

The pH of the sand during growth was about 6.5. The seeds were inoculated with an effective strain of specific nodule bacteria. The plants were grown as described in the first experiment, with sowing 17 March and harvest 4 July. The stages of growth of the harvested plants were as follows: the barley was quite ripe in all pots, peas ripe and partly dry, red clover and lucerne flourishing and still green. The approximate dates of plant development are given in Table VIII.

Table VIII

Plants	Germination	Flowering	Maturity
Barley	24 March	—	3 July
Peas	29 March	15 April	15 June
Lucerne	24 March	3 July	—
Clover	23 March	3 July	—

The experimental results are given in Table IX, and the nitrogen determinations in Table X.

Table IX. *Mean weight of barley (dry matter) per pot*

Series	Total plant		Grain		Root g.	Overall length of roots cm.
	g.	as % of B	g.	as % of B		
A. Barley (10 plants)	3.00 ± 0.33	—	0.55 ± 0.10	—	0.32	32.90
B. Barley (5 plants)	2.87 ± 0.19	100	0.70 ± 0.10	100	0.27	40.30
BC. Barley (5 plants) + peas	4.28 ± 0.41	149	1.40 ± 0.18	200	0.50	49.40
BD. Barley (5 plants) + lucerne	3.18 ± 0.27	106	0.77 ± 0.13	110	0.40	39.03
BE. Barley (5 plants) + clover	2.80 ± 0.16	93	0.70 ± 0.14	100	0.40	39.05

Only in the peas-barley series was there a beneficial effect from mixed cropping, it being shown in that series as an increased yield of barley grain (Pl. VII, fig. 2). Though the roots of barley (in barley-pea pots) were well developed, no interpenetration of root system was observed. In the

other series (lucerne and clover pots) the leguminous plants had no evident effect on the growth of the barley.

Once more, regarding the nitrogen percentage and content of the barley, only in the pots with peas was there a demonstrable effect of the leguminous companion (Table X).

Table X. *Nitrogen percentages and total nitrogen in barley*
(dry matter)*

Series		Nitrogen %	Nitrogen mg. per pot	Nitrogen as % B
A.	Barley (10 plants)	0.55	16.50	—
B.	Barley (5 plants)	0.51	14.64	100
BC.	Barley (5 plants) + peas	0.87	37.24	254
BD.	Barley (5 plants) + lucerne	0.52	16.54	113
BE.	Barley (5 plants) + clover	0.59	16.52	113

* These analyses were made on bulked samples.

III. DISCUSSION

In both experiments peas have been the best companion of rye-grass and barley in their respective years. This beneficial influence of peas, as well as the less well marked effects of red clover and serradella, upon rye-grass, is explainable on the supposition that rye-grass, being a perennial plant, was able to utilize the nitrogenous compounds excreted from nodules of its leguminous companion during its whole season of vegetation.

Tables III and VIII show that in the conditions of these experiments rye-grass was better fitted than barley to make use of the nitrogen provided by a companion crop of peas. The yield of total nitrogen in rye-grass per pot in the peas-rye-grass series was nearly five times as big as the total nitrogen contained in grass grown alone; in the barley-peas series the nitrogen in the barley was only about 2.5 times as great as that of the barley grown in absence of peas.

In a study of root habits of mixed species, Kaserer⁽¹⁾ observed a maximum of root interpenetration when a legume and non-legume were grown together. Thornton and Nicol (see (6)) have made similar, though less extensive, observations. In the experiments described in the present paper, considerable root interpenetration was seen only in the rye-grass-clover pots. Little or no interpenetration of roots was noted in the other series; in spite of that, the associated growth of peas with rye-grass had a better influence upon the development of grass than did the association of clover with rye-grass.

The failure of lucerne and clover to influence the yield and nitrogen

content of barley grown in mixture with them under the conditions of this experiment may perhaps be accounted for as follows: it is known that the blooming period of leguminous plants is also the period of most vigorous fixation of nitrogen by the nodule bacteria; probably the excretion of nitrogenous compounds from nodules is most active at the flowering stage. By the time that in the foregoing experiment red clover and lucerne were in the blooming period, the accompanying barley had already ripened and was unable to utilize the available nitrogenous material. In other words, an excretion of nitrogen compounds from nodule-bearing plants (lucerne and clover) began at a stage of growth of barley that was much too late for the barley to benefit therefrom. The beneficial influence exerted upon barley by peas was evidently due to the circumstance that the period of flowering of peas, and the most vigorous growth of barley, almost coincided.

A non-legume cannot function as indicator plant towards nitrogen if it has reached a stage of growth at which its uptake of nitrogen is small.

In carrying out experiments with mixed crops it is therefore essential to associate species of plants (one leguminous, the second non-leguminous) having approximately parallel and equal periods of vegetation. As an alternative, should the duration of growth of the two companion species differ, the sowing dates should be modified, the plant having the longer period of growth being sown first by a suitably calculated interval. If neither of these precautions is adopted, a mixed crop experiment as a test of the presence of nitrogenous excretions from the leguminous component will have its value seriously reduced or even nullified.

IV. SUMMARY

1. The total yield of dry matter of rye-grass, grown in the presence of inoculated (*a*) peas, (*b*) red clover, (*c*) serradella, in sand with no added nitrogen, after 13 weeks' growth was increased by about three times in the peas-rye-grass series, twice in the clover-rye grass series, and nearly twice in the serradella series in comparison with the yield of rye-grass grown alone.

2. The nitrogen percentage and total nitrogen yield of rye-grass were greatly influenced by associated growth with peas, clover or serradella. Rye grass grown with peas after 13 weeks' growth contained nearly five times, grown with clover three times, grown with serradella about twice, as much total nitrogen, as grass of the same age similarly grown, but in the absence of leguminous plants.

3. Among the three leguminous species tested at Puławy, peas were the best companions for rye-grass, giving the highest amount of assimilated nitrogen, while serradella gave the smallest.

4. In another experiment, when barley and (a) peas, (b) red clover, (c) lucerne were grown together in sand without added nitrogen, only peas exerted a beneficial influence upon the yield of dry matter and the nitrogen percentage and total nitrogen yield of barley.

5. No influence upon barley growth was noted in the red clover-barley and in the lucerne-barley series. This was probably due to the circumstance that the period of most vigorous fixation of nitrogen by clover and lucerne nodule bacteria almost coincided with the period of ripening of barley, and at this stage of growth barley was unable to utilize the available nitrogenous compounds.

6. Rye-grass made a better use than did barley of nitrogen provided by peas grown in association.

7. An extensive root interpenetration in the clover-rye grass pots was noted. There was little or no root interpenetration in the other series of experiments with barley.

8. Certain precautions in the conduct of mixed cropping experiments are adumbrated.

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CXCIII. THE COMPOSITION OF FORAGE CROPS.

I. RYE GRASS. (WESTERN WOLTHS.)

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MANY papers dealing with the composition and digestibility of forage crops are to be found in the literature, but the analyses are usually restricted to those few groups included in the conventional methods of agricultural analysis. These rarely extend beyond the determination of ash, or mineral constituents, crude protein, crude fibre and ether-soluble material. The sum of these analyses is far below 100 % in the case of grasses, and the difference from 100, often 40-60 %, is expressed vaguely as "N-free extractives" or "soluble carbohydrates". In the belief that more information should be obtained about the carbohydrate constituents of forage crops in general, and this ill-defined fraction in particular, detailed analyses have been undertaken using methods which have been developed in the past few years. Not only is it desirable to obtain further knowledge as to the final composition of agricultural materials used as feeding stuffs, but also to be able to follow the changes which take place during development, maturation, drying and storage. This paper records a preliminary study of the development of rye grass and its conversion into hay.

Sampling.

The samples were taken from Pastures Field, Rothamsted, in April, May and June 1935, the seed having been sown under oats the previous season. The selected area, 21 yd. sq., was divided into small plots each $1\frac{1}{2}$ yd. sq. Sampling was effected by cutting with shears an area of 1 sq. yd. within the small plot, this being conveniently determined by the use of a wooden frame of that size. The rejection of margins permitted passage between the areas to be sampled and obviated the necessity of avoiding plots adjacent to one previously sampled on account of possible edge effects. At each time of sampling eight plots, selected at random in the usual way, were cut. The grass from these plots was bulked, again at random, into two samples (A and B), each containing the yield from four plots. In this way an estimate of error due to variation in composition is possible and, since all determinations were subsequently made in duplicate on these A and B samples, an estimate of analytical error has also been obtained. The number of plots cut at each time of sampling is adequate since the purpose of these investigations is a study of composition and not of yield or rate of growth. Many more plots would have to be sampled to obtain reliable figures for the latter.

Treatment of samples.

The grass when cut was bulked into A and B samples and removed to the laboratory. By hand-picking, all weeds and stubble were separated, and the sample placed in an oven at 100° for a short period to inactivate enzymes. Drying was then effected in a drying room at a moderate temperature. After chaffing the samples were ground in a high speed mill to pass 60-mesh.

Other information.

The weather conditions of early summer of 1935 were exceptional, the rainfall in the latter part of May and in June being much above average and the sunshine deficient. Between 26 April, when the first samples were taken, and 21 June, when the last were cut, the rainfall totalled 4.9 in. The sunshine in June, up to the 21st, was 92.8 hours, the average for the period being 143.4 hours. As a result the grass when mature was greener and more stemmy than is usually the case.

The whole area was mown on 22 June and the grass left for hay in the usual way, being turned once. A severe thunderstorm (0.28 in.) soaked it when partly dry. When dry, a large random sample was taken (28 June) so that information might be obtained as to the changes normally occurring in the process of hay making.

Analytical methods.

The following analyses were carried out on the air-dried material:

1. *Ash*: in an electric muffle furnace.
2. *Crude protein*: N by Kjeldahl $\times 6.25$.
3. *Cold water-soluble fraction*: 24 hours at room temperature, nitrogen being determined in the residue.
4. *Crude fibre*: official method.
5. *Total pectin* (expressed as calcium pectate yield)
6. *Cellulose*: Norman & Jenkins [1933].
7. *Total furfuraldehyde yield*: by distillation with 12% HCl and precipitation as the phloroglucide.
8. *Furfuraldehyde* from cellulose.
9. *Lignin* after hydrolysis: Norman & Jenkins [1934, 1], the material being pretreated with alcohol-benzene.
10. *Ether-soluble fraction*: by extraction in a Soxhlet apparatus.

RESULTS.

Some information about the rate of growth of the grass may be obtained from the dry weights of the samples (Table I), each representing the yield from 4 sq. yd.

Table I. *Yield of rye grass from 4 plots each of 1 sq. yd.*

Sample		
1	April 26	A 97.6 B 102.8
2	May 10	A 201.2 B 206.4
3	May 24	A 323.5 B 290.9
4	June 7	A 494.4 B 381.8
5	June 21	A 786.5 B 605.3

Non-structural constituents.

Figures relating to the non-structural constituents are given in Table II and call for no special comment except in the case of the water-soluble fraction, which was very high in the younger samples. More than half of the material of the first three samples was extracted by cold water and of this the protein accounted for only a small part. Hot water was no more effective and indeed removed rather less protein, no doubt owing to coagulation. The nature of the

Table II. *Some non-structural constituents of rye grass.*

All analyses expressed as % on oven-dried samples.

Each figure is the mean of 4 analyses—duplicate determinations on 2 independent composite samples.

Sample period	Ash	Crude protein	Cold water-soluble	Protein in cold water extract	Hot water-soluble	Protein in hot water extract	Ether extract
1	9.52	10.11	53.92	3.17	52.43	3.01	3.46
2	9.11	8.56	52.28	2.90	53.02	2.39	3.42
3	7.84	7.27	54.16	2.23	55.46	2.29	6.34
4	7.66	7.15	46.21	2.45	47.76	2.64	4.12
5	7.50	4.72	37.21	0.89	38.27	0.89	4.84
Hay	7.58	5.46	32.07	2.65	31.29	2.12	2.62
*s.e. \pm (sampling error)	0.30	0.09	0.55	0.12	0.63	0.22	0.15
s.e. \pm (analytical error)	0.19	0.09	0.36	0.10	0.30	0.08	0.44
Analytical error % \pm	2.3	1.2	0.8	4.2	0.6	3.8	10.6

* s.e. = Standard error. Sampling error includes analytical error.

Since each figure in the table is the mean of 4 analyses, the sampling and analytical errors per sample are obtained by doubling the errors given.

non-nitrogenous water-soluble fraction will be dealt with later. The increase in crude protein content on drying for hay was of course due to carbohydrate losses, and the apparent increase in crude protein passing into solution in cold and hot water in the same sample to some protein degradation resulting in the production of soluble products. This will be discussed later.

Structural constituents.

Analyses of some structural constituents are given in Table III. With increasing age the cellulose content rose rapidly, particularly in the samples 4 and 5. For comparison, crude fibre determinations were made by the official method. The deficiencies of the crude fibre figure have been dealt with in detail

Table III. *Some structural constituents of rye grass.*

All analyses expressed as % on oven-dried samples.

Each figure (except N in lignin) is the mean of 4 analyses—duplicate determinations on 2 independent composite samples.

Sample period	Cellulose	Furfuraldehyde from cellulose	Xylan in cellulose	Total furfuraldehyde yield	Furfuraldehyde from polyuronides	Lignin	N in lignin	Crude fibre	Calcium pectate
1	20.89	2.63	4.11	7.29	4.66	4.63	0.29	18.76	0.91
2	20.45	2.75	4.30	7.73	4.98	4.37	0.28	18.17	0.41
3	23.15	3.99	6.19	7.77	3.78	5.08	0.27	17.08	0.52
4	29.69	5.44	8.45	9.85	4.41	6.30	0.27	21.54	0.60
5	36.30	7.09	11.02	11.31	4.22	8.48	0.28	26.34	0.42
Hay	40.85	7.61	11.80	13.15	5.54	9.32	0.28	31.60	0.19
s.e. \pm (sampling error)	0.31	0.14	0.21	0.16	0.24	0.17	*	0.42	0.05
s.e. \pm (analytical error)	0.21	0.09	0.14	0.22	*	0.07	*	0.33	0.04
Analytical error % \pm	0.76	1.92	1.79	2.4	5.3	0.90	*	1.54	7.85

* Not determined.

elsewhere [Norman, 1935, 1] but it is sufficiently apparent from the rye grass samples that the cellulosic structure of the plant is underestimated in an irregular manner in the fibre determination. Whereas in sample 1 the crude fibre figure accounts for about 90 % of the cellulose, in samples 4 and 5 only 72 % is estimated. A contributory factor in this is the increase of xylan in the cellulose with increasing age. This change is more obvious if the xylan be expressed as a percentage of the cellulose containing it (Table IV).

Table IV. *Xylan content of rye grass at different ages. Expressed as % of the cellulose.*

Sample	Xylan
1	19.7
2	21.0
3	26.7
4	28.5
5	30.4

Similar, but not entirely regular, increases in the content of xylan associated with the cellulose have been found in the barley plant by Norman [1933] and by Phillips & Goss [1935]. It is not easy to see why there should be this change in the nature of the cellulosic framework of the plant with increasing age. The glucose and xylose units forming the cellulose and xylan are presumably laid down together by the same mechanism, the balance altering in favour of the pentose as the plant becomes more mature. The pentose metabolism of plants is little understood as yet and provides a number of such problems. It has previously been pointed out that a calculation of total pentosans from the furfuraldehyde yield of the whole material is misleading because of the changing proportion of xylan in association in the cellulose [Norman, 1933] and this fact is again evident from the rye grass results. The total furfuraldehyde yield increased with age, but whilst in the youngest material 26 % of the total was derived from the xylan in the cellulose, the amount from this source increased to 62 % in the mature material. The nature of these changes may be seen from Fig. 1. Since the direct determination of hemicelluloses is unsatisfactory, the furfuraldehyde not from cellulose is taken as a measure of this group of encrusting polyuronides. No correction has been made for the furfuraldehyde derived from pectin, since the pectin content was quite low throughout. The proportion of encrusting polyuronides apparently remained much the same over the whole period of growth. Information as to the actual amount is not given by these analyses. However, the furfuraldehyde yields of hemicellulose preparations that have been obtained indicated that the factor for conversion of furfuraldehyde from this source to polyuronide is a little over 2.

Since the ripening of grass is always said to be accompanied by lignification, and since the extent of lignification considerably affects digestibility, the lignin contents of these samples are of especial interest. The determination of lignin has been a matter of much research lately, and whilst some of the disturbing factors have been recognized, it cannot be said that a method of general applicability has been evolved. In the presence of protein, errors are introduced by the linkage of protein fission products with the lignin [Norman & Jenkins, 1934, 2]. Some workers have corrected for this disturbance by determining the N present in the lignin product, calculating this as protein and subtracting. Such a correction is not justified, and until such time as a method which shall be free from protein interference is devised, it is preferable simply to record the N found in the lignin. The pretreatment advocated by Norman & Jenkins [1934, 1]

for reducing the errors due to the presence of pentose groups does at the same time considerably reduce the protein disturbance. The lignin figures given in Table III were obtained on materials which had been given this pretreatment following extraction with alcohol-benzene, as usually advocated for woods. The apparent lignin residue contained over 6% N on an ash-free basis in the case of the youngest sample. It was curious however that the actual amounts of N retained by the lignin were almost identical in each case although the actual yield of apparent lignin increased progressively with age, the final sample containing

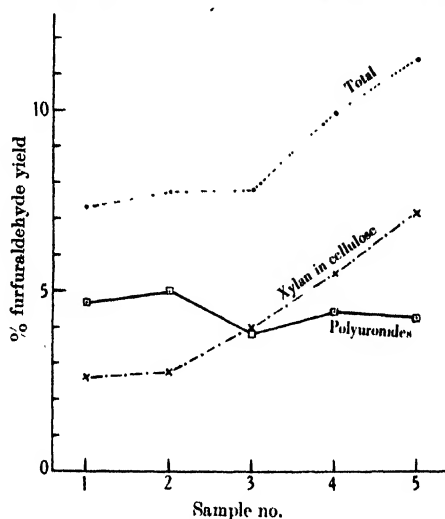


Fig 1. Distribution of furfuraldehyde yield from rye grass samples.

nearly double that of the youngest. The error due to protein may therefore be taken to be more or less constant in this series and the increase in lignin content to be from about 3% in the youngest sample to about 7% in the most mature. The importance of the alcohol-benzene extraction for the removal of fatty and waxy compounds in the case of such green materials was made evident when lignin determinations were carried out on the ether-extracted residues, an acid pretreatment being given in both cases. For comparison, the determination was also made without a solvent extraction (Table V).

Table V. *Effect of solvent extraction on lignin determinations.*

All results expressed on 100 g. oven-dry material.

Rye grass sample	Alcohol-benzene extraction		Ether extraction		No solvent extraction	
	Apparent lignin	N in lignin	Apparent lignin	N in lignin	Apparent lignin	N in lignin
1	4.63	0.29	*	*	6.58	0.25
2	4.37	0.28	5.27	0.33	6.37	0.21
3	5.08	0.26	5.99	0.31	5.72	0.21
4	6.30	0.27	6.79	0.32	7.14	0.20
5	8.48	0.28	9.21	0.28	9.73	0.19
Hay	9.31	0.28	11.15	0.32	10.64	0.20

* Not determined.

It is evident that a further examination of the effect of solvent extraction on the determination of lignin in green plant materials is called for. Although treatment with alcohol-benzene resulted in the lowest yield of lignin residue, the nitrogen in the lignin was actually increased by extraction with either ether or alcohol-benzene. The differences between ether-extracted and alcohol-benzene-extracted samples are greater than could be accounted for by the higher N content of the lignin from the former.

Water-soluble constituents.

The astonishingly high content of material soluble in cold water in these samples led to an investigation of the nature of the substances removed. The major constituent of the extract appears to be a fructose anhydride or laevan. The presence of such fructose polysaccharides in members of the Gramineae has been reported at various times, and recently Challinor *et al.* [1934] have studied the laevan from rough-stalked meadow grass. The yield of pure laevan obtained by them was less than 1% of the dry weight of the grass taken. Buston [1934], in the course of an investigation on the polyuronides of grasses, determined the amount of reducing sugar in the aqueous extract of young grass samples before and after hydrolysis with 1% H_2SO_4 for 40 min. The increases on hydrolysis, by no means all necessarily due to fructose, suggest that the fructosan content of the samples investigated by him was low, except perhaps in Crested dog's tail (*Cynosurus cristatus*). Preparations of fructosan have also been obtained from rye [Kizel & Kretovitch, 1934] and from barley [Archbold & Barter, 1935].

The accurate determinations of the fructosan in the rye grass samples presented difficulties which have not been entirely overcome. It was first determined that an aqueous extract could be hydrolysed readily by low concentrations of acid and that 0.5% oxalic acid gave no increase in fructose after heating for 30–45 min. Even at such a dilution, hydrolysis was extremely rapid in the first 15 min. Determinations were carried out first on cold aqueous extracts of the samples, the extraction being made for 24 hours. This was found to give an incomplete extraction of fructosan and accordingly a second treatment for the same time was given. After clearing with a small amount of basic lead acetate and de-leading with sodium phosphate, the total reducing value was determined by the Shaffer-Somogyi [1933] micro-method, and the aldoses by hypiodite oxidation for 2 hours in a refrigerator. Similar determinations were made after hydrolysing the extract by boiling with 0.5% oxalic acid in a water-bath for 1 hour, conditions which were found to result in the complete hydrolysis of the fructosan. The results are given in Table VI, the figures obtained by the copper method being calculated as fructose and those by the iodine method as glucose.

Table VI. *Carbohydrate in aqueous extracts of rye grass samples.*

Sample	First extraction				Second extraction			
	Total sugar before hydrol.	Total sugar after hydrol.	Apparent glucose before hydrol.	Apparent glucose after hydrol.	Total sugar before hydrol.	Total sugar after hydrol.	Apparent glucose before hydrol.	Apparent glucose after hydrol.
1	3.10	27.47	9.66	9.96	0.06	0.98	0.56	0.70
2	7.00	30.88	8.12	9.18	0.11	0.77	0.07	Trace
3	6.37	35.15	9.20	10.49	0.05	1.16	0.50	0.56
4	4.28	26.66	7.97	8.50	0.11	1.06	0.47	0.42
5	6.54	21.77	7.02	6.69	0.12	1.09	0.47	0.47
Hay	5.60	15.68	7.83	7.83	0.07	0.34	0.50	0.25

Two important facts are at once apparent. First, the increase in reducing sugar produced by hydrolysis is due almost solely to the liberation of fructose, since the apparent glucose figures show little difference after hydrolysis. Second, the extract must contain oxidizable material unaffected by the copper reagent, but easily oxidized by hypiodite, because the total reducing value by the copper reagent is far lower than the apparent glucose content by hypiodite oxidation. This is, of course, a common observation with plant extracts and makes it impossible to get true figures for fructose and fructosan contents by this method. Hot water extraction for 1 hour gave figures for total reducing sugar very similar to the sum of the cold extracts with the difference that the amounts of apparent glucose after hydrolysis determined by hypiodite oxidation were a little higher. Direct hydrolysis of the fructosan in the grass was effected by heating for 30 min. with 5% oxalic acid in a water-bath. As far as could be judged, the results obtained indicate that hot aqueous extraction or repeated cold aqueous extraction does result in complete removal of the fructosan. To avoid difficulty due to the presence of aldose sugars and non-sugar reducing substances, determinations were carried out by the copper reagent on extracts which were first oxidized by hypiodite, the excess iodine being removed with sulphite. The procedure adopted was similar to that recently described by Van der Plank [1936]. The grass samples, finely powdered, were extracted for 30 min. in a boiling water-bath, and a portion of the extract, after clarification, hydrolysed by boiling with 2% oxalic acid for 30 min.

5 ml. portions of the unhydrolysed and hydrolysed extracts were treated with 2 ml. *N*/10 iodine (containing 34 g. KI per litre) and 1 ml. *N*/4 NaOH and kept in a refrigerator just above freezing point for 2 hours. After acidification with 1 ml. *N*/3 H₂SO₄ the excess iodine was removed by titrating with dilute sulphite. The reducing value of this solution was then determined in the usual way with the Shaffer-Somogyi reagent. As a result of the pretreatment with hypiodite, considerable quantities of iodide are present which appreciably affect the apparent reducing value of fructose. The values for the fructose content must therefore be taken from a curve specially determined for the quantity of iodide that is present. Unless this be done the error introduced is over 10%. The results obtained are given in Table VII. The fructose or fructosan present passed

Table VII. *Fructosan in rye grass samples.*

Results calculated on the basis of 100 g. oven-dry grass.

Sample	Before hydrolysis as fructose	After hydrolysis as fructose	Fructosan as fructose
1	1.06	28.21	27.15
2	3.00	26.62	23.62
3	3.10	37.53	34.43
4	2.69	25.33	22.64
5	3.73	18.87	15.14
Hay	5.23	14.19	8.96

through a sharp peak and then rapidly declined as maturity approached. A further considerable fall took place in the process of hay making. The intervals at which the samples were taken were not sufficiently short to provide a full picture of the changes in the fructosan content. It is however quite evident that this polysaccharide must be the chief carbohydrate reserve of rye grass, and, because of the high availability of fructose, must be also of great nutritional importance. The method of drying adopted was such that some slight hydrolysis of fructosan to fructose might take place. A more detailed investigation of the

distribution of this polysaccharide is in progress. Young wheat plants have also been found to contain fructosan, the amount decreasing rapidly as the ears form (unpublished).

Hydrolysis of rye grass fructosan.

A crude preparation of fructosan was obtained from an aqueous extract of young dried rye grass (collected 17 May). Proteins were removed with basic lead acetate and lead with phosphate. After concentration to a small volume *in vacuo*, inorganic salts were precipitated by the addition of alcohol to give a concentration of 47%. After further concentration, the solution was poured into a large excess of absolute alcohol giving a creamy white precipitate, which was immediately filtered off. Purification of the precipitate was attempted by several means, including the baryta fractionation described by Archbold & Barter [1935], but no method was very successful. The crude precipitate had a specific rotation $[\alpha]_D -40^\circ$ approximately and very slight reducing properties. After hydrolysis for 15 min. with 0.5% oxalic acid the sugar produced amounted to 87% on an ash-free basis, 84.4% being fructose. This polysaccharide was very susceptible to hydrolysis and yields of sugar of a similar magnitude were obtained on heating for 30 min. with 0.05% oxalic acid; using 0.025% oxalic acid the sugar produced did not exceed 58%. Slight hydrolysis was effected on prolonged boiling with water, 19.0% sugar being obtained after 1 hour and 20.8% after 3½ hours.

The changes occurring during hay making.

No direct determinations of the total losses occurring during hay making were made, as the whole area was cut. Some indication of the magnitude of these losses may, however, be obtained by comparing in the mature sample and the hay the percentage contents of constituents such as cellulose and lignin which would be unlikely to be reduced by the treatment. Such a comparison suggests that the losses were in the neighbourhood of 10%. Of this more than half was fructosan, presumably as a result of respiration, or enzymic conversion of the polysaccharide into fructose which would be liable to be washed out by rain. As evidence of the breakdown it is significant that the hay contained the highest percentage of free fructose. Some losses of ash and ether-soluble material also occurred. There does not appear to have been any loss in the nitrogenous constituents as judged by the total N figures, but extensive changes must have taken place because the cold water-soluble N increased from approximately 20 to 50% of the total. The xylan in the cellulose was slightly reduced, with a concurrent increase in furfuraldehyde from the polyuronides. The pectin content, though low in the mature grass, fell sharply.

DISCUSSION.

The system of analysis described in this paper though not complete accounts far more satisfactorily for the various constituents of grass than conventional methods. It is imperfect in that a reliable method for the determination of the polyuronide hemicelluloses has not been found. Buston [1934] attempted this determination by direct preparation and found the apparent hemicellulose content of various grasses by this method to be 16–20%. His procedure is, however, very laborious and is open to certain criticisms [Norman, 1935, 2], the chief perhaps being that any alkaline extraction also removes cellulosan from the cellulose, with the result that the product does not only consist of encrusting polyuronides. An attempt to avoid this difficulty will be described elsewhere.

In the case of sample 5, only 43.4 % of the grass is accounted for by the conventional methods, leaving 56.6 % to be described as soluble "carbohydrates". Excluding any estimate of the hemicelluloses, the analyses given in this paper on the same sample total to over 80 %. The presence of a considerable amount of soluble carbohydrates in the form of fructosan is now established and the role of this in nutrition will have to be examined. Presumably it is readily utilized by the animal, though the action of digestive enzymes on this polysaccharide has not been studied. Archbold & Barter [1935] found that their fructosan preparations were but slowly attacked by invertase. The position of fructosan in the metabolism of the plant can as yet be only a matter of speculation. In the young plant fructosan is produced more rapidly than cellulose, but later cellulose production predominates. This is shown in Table VIII in

Table VIII. *Amounts of various constituents as percentages of that present in final sample (5).*

Sample	Dry matter	Fructosan	Cellulose	Lignin	Crude protein
1	14.4	22	8	8	31
2	29.4	41	17	15	54
3	44.1	87	28	26	69
4	63.0	84	52	48	96

which the chief constituents in each sample are expressed approximately as percentages of that present in the final sample, the increases in yield being taken from the means of the figures in Table I.

Some of the advantages that have been ascribed to the higher protein content and greater digestibility of the cellulose of young grass may in fact be due to its much higher content of fructosan, if other species of grass are at all similar to rye grass.

SUMMARY.

1. Samples of rye grass cut at fortnightly intervals have been analysed, particular attention being given to the structural constituents.

2. The contents of cellulose and lignin increased rapidly as maturity approached. The percentage of xylan in the cellulose also increased with age. The polyuronide hemicelluloses, as judged by furfuraldehyde yield did not exhibit any regular increase and were lower in the mature grass than in the young grass.

3. A water-soluble fructosan, or laevan, was found in considerable amounts in the younger samples, reaching at one stage over 37 % expressed as fructose. The percentage content of this polysaccharide fell rapidly on maturity.

4. Losses in hay making were of the order of 10 %, mostly accounted for by loss of fructosan.

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CXCV. THE COMPOSITION OF FORAGE CROPS
II. RYE GRASS (WESTERN WOLTHS). CHANGES IN
HERBAGE AND SOIL DURING GROWTH

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PRELIMINARY experiments on the developmental changes in composition of rye grass revealed some interesting features, not the least of which was the presence of a water-soluble fructosan in considerable amount in the young grass [Norman, 1936]. A more detailed investigation involving weekly samples of both soil and herbage throughout the growing season has now been undertaken. In the soil analyses the more mobile forms of nitrogen and carbon were studied, since the changes in total nitrogen and carbon during the growth of a single crop of rye grass are likely to be so small as to lie within the experimental error of the determinations. The ammonia and nitrate contents of the fresh soil were measured, and also the ammonia, nitrate and carbon dioxide produced when the soil samples were incubated under standard conditions. Methods of this type have been extensively used here to study the influence of cropping on the soil organic matter [Orchard, 1933; Richardson, 1935; Marshall, 1936]. The ammonia and nitrate in the fresh soil show the amount of nitrogen immediately available to the crop, while the incubation values give an index to the amount of readily decomposable nitrogen and carbon in the soil organic matter.

Methods employed for the grass

The samples of grass were taken from Hoosfield, Rothamsted, from an area under barley in the previous season. The selected area was laid out with 98 plots each $1\frac{1}{2}$ yd. sq. As before, an area of 1 sq. yd. was cut from the plot at the time of sampling. Each sample consisted of 6 sq. yd. taken at random, and bulked. Owing to the variation in the density of the herbage this was insufficient to give reliable figures for increase in dry weight. The bulked samples were treated as in the previous season.

Soil sampling was commenced on 25 April 1936, $2\frac{1}{2}$ weeks before the first grass sample was taken. On 4 May a light dressing of sulphate of ammonia was applied at the rate of $1\frac{1}{2}$ cwt. per acre. The stand of grass was rather thin, and the grass tended to be of a stemmy character. The season was almost uniformly wet, a circumstance which favoured the later growth of clover on the plots which had been cut earlier in the season. A sample was cut and left for hay at the time when the grass was judged by the farm manager to be ripe for this purpose.

The analytical methods employed for the grass were as described in the previous paper [Norman, 1936].

A. COMPOSITION OF RYE GRASS

The approximate rate of growth of the rye grass may be seen from Table I. These figures do not fall on a smooth growth curve because insufficient plots

Table I. *Yield of rye grass per sq. yd. (first cut)*

Sample	Date	Weight oven-dry g.	Sample	Date	Weight oven-dry g.
1	12 May	23	8	30 June	161
2	19 "	54	9	7 July	161
3	26 "	64	10	14 "	165
4	2 June	93	11	20 "	151
5	9 "	126	12	26 "	128
6	16 "	166	13	21 Sept.	—
7	22 "	150			

were cut to even out the irregularity in the density of the grass, but they leave little doubt that there was no increase in dry weight per unit area after mid-June.

Non-structural constituents (Table II)

The changes observed in ash and crude protein present no special features, and are of the type usually observed in a growing crop. The anomalous figure for ash in sample 4 was due to the presence of some clay through sampling under

Table II. *Some non-structural constituents of rye grass*

All analyses expressed as % on oven-dried material

Sample	Ash	Crude protein	Cold water- soluble	Protein in aqueous extract	Fructose before hydrolysis	Fructose after hydrolysis
1	9.8	11.7	49.5	3.3	2.1	25.6
2	8.5	9.3	52.5	4.4	3.1	26.9
3	8.1	9.1	56.9	4.7	3.6	30.1
4	(10.3)	7.7	46.5	4.1	1.8	28.1
5	5.5	6.9	44.4	2.6	3.2	26.8
6	5.5	6.4	39.0	1.9	2.1	21.3
7	6.9	6.0	31.5	2.0	2.4	17.0
*Hay	6.4	5.6	26.4	2.9	5.1	11.8
8	7.0	5.4	30.7	2.2	2.3	14.6
9	6.6	4.7	25.1	1.6	2.0	12.6
10	6.8	4.7	20.7	1.5	1.0	7.0
11	7.5	4.2	15.7	1.2	1.0	5.7
12	7.9	5.1	15.7	1.6	0.9	3.2
13	5.4	6.7	9.9	2.1	0.1	0.4
†s.e. of mean ±	0.10	0.09	0.23	0.07	0.09	0.17

* Cut with Sample 7; left 1 week in field.

† The standard errors of these determinations are calculated on all samples, and include those in Table V.

bad weather conditions. As observed previously there is a high percentage of water-soluble material in this grass in its younger stages, only a small portion of which is nitrogenous. The major part is fructosan, which constituent rises to a peak towards the end of May subsequently falling sharply as the grass matures and becomes senescent. The free sugar consists largely of fructose, but no particular significance is to be attached to the amounts found, since this is believed to be in part a result of the drying procedure. In Sample 3, when

dried, 3.6 % free fructose was found. Grass cut at the same time and extracted when fresh with hot 95 % alcohol contained only 1.64 % total reducing sugar, expressed as fructose, of which 1.57 % was fructose. The peak content of fructosan occurred while the dry weight was still rapidly increasing, so that this polysaccharide must be of the nature of a temporary reserve. The peak was appreciably lower than that recorded in the previous season and coincided approximately with the time of full emergence of the head.

The distribution of fructosan within the plant at the time of the fourth sampling was studied (Table III). It appears that the stem is the chief place

Table III. *Distribution of fructosan in rye grass. Sample 4.*
Cut 2 June

Region	Proportion of aerial parts	% free fructose	% fructose after hydrolysis
Leaves	25.4	1.0	24.4
Stems	51.5	1.9	35.4
Heads	23.2	1.5	17.3
Root	—	0.7	25.0

of storage. Division of the stem into internodes showed that the first internode above ground level is richest in this constituent, 42.8 % being found. In the second internode only 34.1 % was present.

The fructosan, soluble protein and soluble ash constituents do not together account for all the cold water-soluble material, and evidence of the presence of a glucose polysaccharide, hydrolysed considerably less easily than fructosan, has been obtained. The satisfactory determination of a mixture of these two polysaccharides has not yet been solved, partly because the conditions necessary for complete hydrolysis of the glucosan result in some destruction of fructose. The new polysaccharide is present in considerably smaller amounts than the fructosan. In sample 3, which contained 30.1 % fructosan, about 6.7 % glucosan was detected.

Structural constituents (Table IV)

The crude fibre determination has been abandoned on the grounds that it is both inaccurate and misleading [Norman, 1935], and reliance is placed instead on the direct determination of cellulose and lignin. The cellulose content increases steadily and rapidly while the dry weight of the plant is increasing and the grass is approaching maturity. The largest percentage increase between the time of the 6th and 7th samples does not in fact represent a spurt in cellulose production, but is partly the result of a fall in other constituents, as may be seen later in Fig. 1 (p. 1560) in which the cellulose present is shown on a basis of g. per sq. yd. After the point at which no increase in dry weight occurs, the percentage of cellulose still rises, though less rapidly, presumably by conversion of some other constituent. There is a limit to this change, for the extremely old grass left till September, Sample 13, is not much richer in cellulose than that cut in late July.

The yield of furfuraldehyde from the cellulose, and therefore the xylan associated with the cellulose, does increase with age, but not in any very steady manner. When the xylan is calculated as % of the cellulose, there is an indication of a tendency to increase during the period of active growth, as was observed in the experiments of the previous year, and then a decrease during senescence. The changes are somewhat irregular as were those found for the barley plant by Phillips & Goss [1935].

Table IV. *Some structural constituents of rye grass*

All analyses expressed as % of oven-dried material

Sample	Cellulose	Furfuraldehyde from cellulose	Xylan in cellulose	Total furfuraldehyde	Furfuraldehyde from polyuronides	Lignin	N in lignin
1	26.1	4.3	6.7	7.4	3.1	3.6	0.3
2	26.4	4.5	7.0	8.8	4.3	4.7	0.3
3	28.9	5.4	8.4	9.1	3.7	5.2	0.3
4	32.0	5.5	8.6	9.8	4.3	5.9	0.3
5	32.2	6.0	9.3	10.9	4.9	7.0	0.3
6	33.8	5.9	9.2	11.4	5.5	8.7	0.3
7	40.4	6.1	9.5	12.4	6.3	9.0	0.3
Hay	43.2	6.4	10.0	13.3	6.9	10.4	0.2
8	38.3	6.3	9.8	12.6	6.3	9.1	0.3
9	39.9	6.3	9.8	13.4	7.1	9.6	0.3
10	43.2	6.2	9.7	13.8	7.6	10.5	0.3
11	47.1	6.0	9.4	14.3	8.3	11.2	0.2
12	46.2	6.3	9.8	14.1	7.9	11.1	0.3
13	48.8	6.4	10.0	15.1	8.6	16.4	0.3

*s.e. of mean \pm 0.25 0.09 — 0.12 — 0.10 —

* The standard errors of these determinations are calculated on all samples and include those in Table VI.

No satisfactory direct method for the evaluation of the polyuronide hemicelluloses has yet been devised, and accordingly the difference between total furfuraldehyde yield and that from the xylan in cellulose is taken as a measure of this constituent. The changes appear to be similar to those exhibited by the cellulose, namely a rapid increase during the period of active growth and subsequently a more gradual increase during the senescent period.

The presence of lignin in forage crops is believed to affect digestibility adversely, and the "running off" of grass is always said to be a result of lignification. The results obtained indicate that the deposition of lignin is a gradual process and not a change which occurs especially at maturity. The figures slightly over-estimate the lignin present, because of the interference produced by the presence of protein, as a result of which the lignin residue contains some nitrogen. No satisfactory correction is applicable nor can the disturbance be wholly overcome. It will be seen that the nitrogen retained by the lignin was almost uniform, and presumably therefore the error is nearly constant. The extremely old grass sample taken in September was considerably higher in lignin than any of the mature samples. Lignin formation seems to continue when other activities have practically ceased.

Taken together, the main structural constituents, cellulose, hemicelluloses, and lignin increase progressively with age. If a rough estimate of the hemicellulose content is made by taking twice the furfuraldehyde figure, it may be said that in the youngest sample these structural constituents account for not less than 36 % of the dry weight; in Sample 7, at maturity, not less than 62 %; and in Sample 11, late in July, not less than 75 %.

Changes in haymaking

The method of sampling did not permit of a satisfactory estimate of the loss occurring during haymaking, but a comparison of the contents of cellulose and lignin in the hay and the appropriate sample cut at the same time suggests that the loss was about 10 %. This seems to be due to removal of some ash,

protein and fructosan. At the same time the proportion of nitrogen soluble in cold water increased, as did also the free fructose, presumably by enzymic hydrolysis of the fructosan.

Some physiological aspects

The presence of the polysaccharide fructosan as a major constituent of young grass, and its disappearance on maturity, raises certain physiological questions which cannot yet be fully answered. Unlike the cereals, which also contain fructosan in the young plant, rye grass does not store any very large amount of carbohydrate in the seed, and instead of being utilized by transformation into starch, as is conceivably the case in cereals, the fructosan in the grass is presumably utilized for general development. There is a strong indication that the structural constituents and, in particular, cellulose, which continue to increase after the dry weight of the plant has reached its maximum, do so at the expense of the stored fructosan. This may be seen from Fig. 1 in which are plotted the

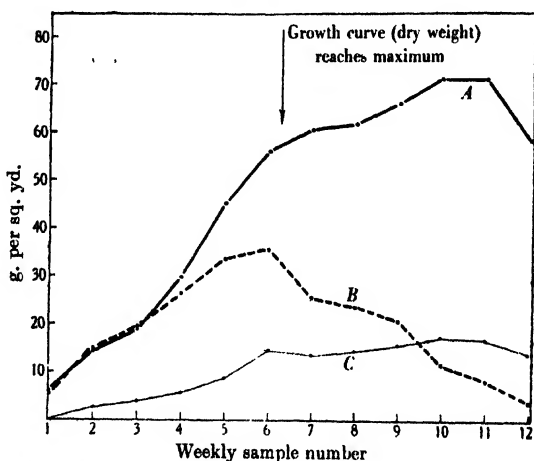


Fig. 1. Cellulose, fructosan and lignin contents of rye grass expressed on an equal area basis.

•—• Cellulose. ■---■ Fructosan. — Lignin.

cellulose, lignin and fructosan in the grass from 1 sq. yd. The approximate point at which the growth curve reached its maximum, after which there was no increase in dry weight, is indicated, and it will be seen that the subsequent fall in fructosan is in part balanced by a rise in cellulose. Since there are no direct figures for hemicelluloses these cannot be plotted, but nevertheless the indication from furfuraldehyde yield is that they too increased concurrently.

Fig. 1 shows also that on an area basis the fructosan maximum occurs later than the peak in percentage content owing to the increase in dry weight. The amount present at the time of taking Sample 6 was nearly $3\frac{1}{2}$ cwt. per acre.

Various factors connected with the formation and utilization of fructosan remain to be elucidated, including seasonal and manurial effects.

Composition of second growth

Certain of the sets of plots were recut later in the season in order to obtain information as to the composition of second growth of the herbage. As a result of the wet summer, clover and weeds in some cases provided serious competition

to the new growth. These experiments are only of a preliminary nature as the series is incomplete, and the samples represent material cut at increasing intervals from sets of plots themselves not originally cut at the same date. The analyses for non-structural constituents are given in Table V. The crude protein

Table V. *Some non-structural constituents of second growth rye grass*

All analyses expressed as % of oven-dried material								
Sample	Date cut	Weeks after first cut	Ash	Crude protein	Cold water-soluble	Protein in aqueous extract	Fructose before hydrolysis	Fructose after hydrolysis
1-1	15 June	5	9.2	10.5	33.0	3.4	2.2	14.2
2-1	29 "	6	9.0	7.5	29.7	2.6	2.5	12.2
3-1	14 July	7	7.4	7.1	27.4	2.3	1.7	8.9
4-1	27 "	8	7.5	5.4	20.3	1.6	1.3	5.5
5-1	11 Aug.	9	8.8	6.7	18.0	1.9	1.2	6.1
7-1	11 "	7	8.2	8.0	26.9	2.2	1.8	4.6
6-1	18 "	9	8.9	7.8	21.3	2.0	1.1	5.1
7-2	25 "	9	8.4	8.2	24.4	3.1	1.6	6.5

content was inclined to be higher than on first growth grass of approximately the same stage of development. The most interesting feature is the low percentage of cold water-soluble material and of fructosan, relative to the protein content. In first growth grass with a crude protein figure of 7.7 % (Sample 4), over 46 % was water-soluble, out of which 28 % could be accounted for by fructosan. In the second cuts of approximately equal or higher protein contents, these figures are not approached. The comparison of Sample 3-1 with 7-1, and Samples 5-1 and 6-1 with 7-2, groups respectively of equal age, shows that considerable differences in composition may result according to the period over which the growth takes place. This is not surprising since equal age does not necessarily imply an equal stage of development, which may be affected by many factors.

A low percentage of water-soluble constituents implies more of the structural cell wall substances, as is observed (Table VI). Cellulose, lignin and polyuronide hemicelluloses are all higher on these samples than on first growth of approximately similar protein content. For example, Samples 4 and 2-1 are almost equal in crude protein, but the sum of cellulose + lignin in the former amounts to 37.9 % and in the latter to 48.0 %. Similarly with Samples 5 and 5-1, the totals are 39.2 and 52.2 %.

Table VI. *Some structural constituents of second growth rye grass*

All analyses expressed as % of oven-dried material							
Sample	Cellulose	Furfur-aldehyde from cellulose	Xylan in cellulose	Total furfur-aldehyde	Furfur-aldehyde from polyuronides	Lignin	N in lignin
1-1	38.4	6.0	9.4	12.2	6.2	8.2	0.4
2-1	37.9	5.9	9.2	12.0	6.1	10.1	0.4
3-1	33.2	5.7	8.9	11.5	5.8	9.2	0.3
4-1	42.9	5.8	9.0	13.3	7.5	11.5	0.2
5-1	40.6	6.0	9.4	12.9	6.9	11.6	0.3
7-1	36.4	6.0	9.4	11.5	5.5	10.0	0.3
6-1	38.4	5.8	9.0	12.1	6.3	11.0	0.3
7-2	39.2	5.7	8.9	12.3	6.6	10.4	0.4

Because of the incompleteness of the series it is impossible to generalize from these results, but they give a strong indication that second growth grass is

more "fibrous" than the first growth; that is to say the amount of structural material present is higher, and the amount of water-soluble constituents, such as fructosan, considerably lower, when considered on the basis of equal protein contents, as is the case when feeding value is in question. If this is applicable to grasses in general it may be the explanation of the belief held by many farmers that autumn grass is not so satisfactory as spring grass even though the nitrogen percentage may be the same. From the analyses obtained the carbohydrates of the second growth would be likely to be less available than those of the first growth.

B. CHANGES IN THE SOIL DURING THE GROWTH OF RYE GRASS

The soil was the heavy clay-loam with flints of Rothamsted; small lumps of chalk were present, and the reaction was pH 8.0. Because the results obtained in studies of the mobile forms of nitrogen and carbon are highly susceptible to changes in the working conditions, the methods employed in the present investigation are described rather fully.

Sampling and analysis

The depth of sampling was 20 cm., a half-cylindrical sampling tool taking a core of 60-70 g. being used. It was pushed in beyond the depth required, and the bottom few cm. of soil discarded. Four samples, each consisting of 6 or 7 cores, were collected in stoppered bottles on each date of sampling. At first the samples were taken entirely at random; later, when the regular sampling of the herbage began, two of the samples were taken from the six plots whose herbage had just been cut, and the other two samples from the plots cut a week earlier. Each of the four samples was analysed or incubated separately, so that the standard error of the determinations gives a combined measure of errors of sampling and analysis.

In the laboratory the soils were broken up by hand, roots and flint fragments being removed, and after thorough mixing the soils were subsampled by quartering. 100 g. were returned to each bottle for the preparation of an extract, 100 g. were transferred to flasks for incubation, 50 g. were used for dry matter determination (24 hr. in electric oven at 105°) and the remainder was air-dried, ground and sieved.

The extracts for ammonia and nitrate determination were made by Olsen's method [1929], slightly modified. 100 ml. of 2M KCl solution were added to 100 g. soil, then enough 2N HCl to give the extract a pH of about 1.0 (to thymol blue), and water to make the total volume of liquid added 200 ml. A few drops of toluene were added as a preservative, and the whole was shaken in a reciprocating machine for 1 hr. The suspension was filtered through a fluted filter (24 cm. diam.), the whole being poured in at once, and the first 25 ml. of filtrate discarded. This stage was always reached on the day of sampling: the filtered extract could be kept several days if necessary in a stoppered flask in a refrigerator.

Ammonia was determined by distilling 100 ml. of the extract, to which 200 ml. of water had been added, from ca. 3 g. of freshly ignited MgO; the distillate was collected in N/50 acid and titrated to an end-point of pH 4.8 with bromocresol green as indicator.

Nitrate was determined in the same solution by further distillation after adding ca. 2.5 g. of powdered Devarda's alloy, and water to replace that lost in the ammonia distillation. Distillation was slow because of frothing, and this gave sufficient time for reduction of the nitrate.

Blanks were determined on all reagents, and the results of the determinations, after blank correction, were calculated on the basis of oven-dry soil.

Ammonia and nitrate in the fresh soil

The soil was first sampled on 25 April, when the grass was still fairly short. It was sampled again on 7 May, 3 days after the application of sulphate of ammonia; then on 11 May; and subsequently at approximately weekly intervals, usually on the dates of cutting the herbage, until 28 July. The results appear in Table VII: for ammonia, the standard error is given at each sampling date because of its variation with time; for nitrate, where the standard error was more constant, a single mean value is given.

Table VII. *Ammonia and nitrate nitrogen in fresh soil*

Sampling date	mg./kg. dry soil														
	Apr. 25	May					June					July			
Ammonia N		7	11	14	19	26	3	9	16	22	30	6	14	20	28
(mean of 4)	1.0	3.7	3.0	4.4	2.2	2.5	3.7	1.9	2.6	1.3	1.1	1.0	1.0	1.2	1.0
s.e. of mean \pm	0.06	1.00	0.33	1.46	0.58	0.53	1.07	0.60	1.76	0.06	0.23	0.07	0.07	0.08	0.13
Nitrate N															
(mean of 4)	0.3	0.2	2.3	0.7	0.3	0.1	1.3	1.1	0.2	0.5	0.9	0.5	0.4	0.6	0.5
s.e. of mean								± 0.24							

Since the nitrogen applied as sulphate of ammonia on 4 May was equivalent to approximately 18 mg./kg., the results for soil ammonia show that there was rapid disappearance of most of the added nitrogen. A similarly rapid disappearance in late spring has been recorded on other grassland soils [Richardson, 1932; Eggleton, 1934]. A small amount of extra ammonia was found in the soil until mid-June; this ammonia was unevenly distributed, as may be seen from the high standard errors over this period, and its presence is attributed to the rather thin stand of grass leaving small "pockets" of unabsorbed ammonia in the soil. Very little nitrate was found, the maximum being 2.3 mg. N/kg. a week after adding the sulphate of ammonia. Any nitrate formed would rapidly be taken up by the herbage, but it is probable that much of the added nitrogen was taken up directly as ammonia. The herbage showed a visible response in colour and growth a week after the sulphate of ammonia application, which agrees with the rapid disappearance of ammonia from the soil in suggesting direct uptake without the necessity of nitrification as an intermediate stage [cf. Eggleton, 1935].

The average levels of ammonia and nitrate before adding sulphate of ammonia and after its effect had disappeared were very low, of the order of 1.0 and 0.5 mg. N/kg. respectively. It has been shown elsewhere [Richardson, 1935] that equilibria exist in the ammonia and nitrate contents of grassland soils; the equilibrium levels in the young grassland soil of the present experiment were lower than those observed in older grassland soils.

Incubated soils

For incubation, 100 g. of fresh soil from each sample were placed in a 350 ml. flask, along with a short test-tube containing 12 ml. of N KOH, and the stoppered flask was placed in an incubator at 30°. On the following day, when the moisture content of the fresh soil had been determined, sufficient water was added to make up the moisture content to 20% on a dry soil basis. Air was blown into the flasks at 2-3-day intervals to maintain the oxygen supply; blank

flasks containing KOH tubes were similarly treated, but the correction necessary for CO₂ in the air was small. After 3 weeks' incubation the flasks were removed, and absorbed CO₂ was determined in the KOH by double titration, first to pH 8.4 (phenolphthalein) and then to pH 3.9 (bromophenol blue) [Orchard, 1933]. Ammonia and nitrate were determined in the soil by the procedure already described. The results are given in Table VIII.

Table VIII. *Ammonia and nitrate nitrogen in incubated soil; carbon dioxide produced during incubation*

Sampling date	...	April	May					June					July 6
Ammonia N, mg./kg. (mean of 4)		25	7	11	19	26		3	9	16	22	30	
		0.9	2.2	1.0	0.5	0.7		1.1	0.4	0.4	0.8	0.6	0.6
s.e. of mean								±0.18					
Nitrate N, mg./kg. (mean of 4)		10.3	14.5	12.0	11.7	9.1	10.6	9.6	10.0	5.5	6.7	8.2	
s.e. of mean							±1.11						
Carbon dioxide C, mg./kg. (mean of 4)		364	292	274	295	314	358	338	328	342	307	277	
s.e. of mean							±15.8						

The soil nitrified readily on incubation, with the result that the ammonia values were below those in the fresh soil. The highest ammonia content was only 2.2 mg. N/kg. in the sample of 7 May, taken 3 days after sulphate of ammonia application. There was a significant rise in nitrate content on 7 May, after the addition of sulphate of ammonia, and then a tendency to decrease: for some time the values remained about the level of the initial sample, but at the end of June there was a fall to values significantly below that level.

Carbon dioxide production was very high by comparison with nitrate production, and whereas nitrate increased in the samples taken after sulphate of ammonia application, carbon dioxide decreased. Throughout May it was significantly below the initial value; it rose to a level nearer the initial value during June, but fell again significantly below it at the end of June and the beginning of July.

Some of the fluctuations in the ammonia and nitrate contents of the incubated soils were clearly due to the presence of the nitrogen added as sulphate of ammonia. To eliminate this, the mineral (ammonia plus nitrate) nitrogen found in the fresh soils was subtracted from that in the incubated soils, the difference, known as the "mineralizable" nitrogen, representing the nitrogen made available by the breakdown of soil organic matter during incubation.

The values for mineralizable nitrogen are shown in Fig. 2, the carbon produced as carbon dioxide during incubation being included for comparison. Both curves show fluctuations of the type commonly observed in microbiological studies of the soil, but there are some clearly marked tendencies. The mineralizable nitrogen was decreasing during most of the period of sampling. Such a decrease during the late spring has been observed in other grassland soils [Richardson, 1935] and attributed to a seasonal rhythm in the soil organic matter. As the soil warms up in the spring, the readily decomposable nitrogen in the soil organic matter is mineralized and taken up by the herbage, leaving less mineralizable nitrogen to appear on incubation. The mineralizable nitrogen was low throughout, by comparison with the level found in older grassland soils.

The carbon dioxide values, which may be taken as an index to the amount of readily decomposable carbohydrate material in the soil, show a fall after sulphate of ammonia addition that was probably due to an accelerated breakdown of carbohydrates in the field. Then there was a rise, attributable to the production of root material rich in carbohydrate by the rye grass. Once this ceased, about mid-June (22 June), there was a fall in both carbon dioxide and mineralizable nitrogen. The peak corresponded roughly with that of maximum content of fructosan.

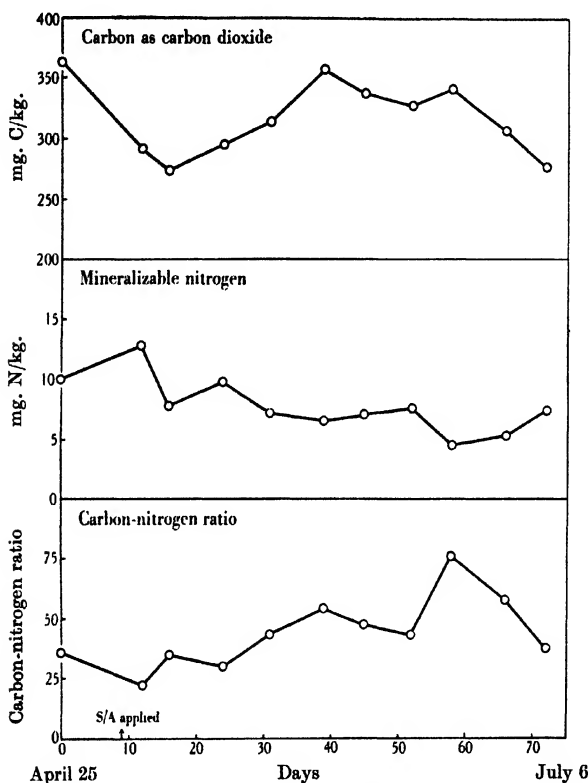


Fig. 2. Changes in the incubation products of rye grass soil. (Note that the vertical scales are different for each of the curves.)

Carbon dioxide and mineralizable nitrogen values frequently appeared to move in opposite directions, as would be expected if some mineral nitrogen was locked up by the micro-organisms that broke down carbohydrates. The major changes in carbon and nitrogen level were not, however, always opposed, and the correlation of the two sets of values, although negative, was not significant ($r = -0.2376$).

The ratio of carbon produced as CO_2 to mineralizable nitrogen, which gives a general index to the condition of the soil organic matter, is also shown in Fig. 2. After a slight decrease resulting from the sulphate of ammonia treatment there was a general rise until the time of cutting hay; evidently the roots, like the shoots, were becoming relatively richer in carbohydrates and lower in nitrogen

content, so that their residues gave a wider carbon-nitrogen ratio in the products of incubation. The cause of the fall in the ratio after 22 June is not so clear; we owe to Dr Nicol the suggestion that there may have been some "resorption" of nitrogen back into the roots from the above-ground parts of the plant.

The soil samples taken from plots cut a week before sampling contained, on the average, slightly more ammonia and gave more nitrate and carbon dioxide on incubation, but the differences from the freshly cut plots were not significant.

SUMMARY

1. The changes in the composition of rye grass cut at weekly intervals throughout the season have been studied and at the same time the amounts of the more mobile forms of nitrogen and carbon in the soil have been followed.

2. The presence of a water-soluble fructosan was confirmed; this constituent increased to a peak of 30.1 % at about the time of full emergence of the head, thereafter decreasing with maturity.

3. The fructosan accumulates chiefly in the stem and appears to act as a temporary reserve, later being utilized for the production of structural constituents.

4. The second growth of grass contained a higher proportion of structural constituents and less fructosan than the first growth when compared on a basis of similar protein content.

5. Ammonia and nitrate determinations in the fresh soil showed rapid disappearance of most of the nitrogen added as sulphate of ammonia, with very little production of nitrate.

6. The determination of ammonia, nitrate and carbon dioxide produced by incubated soil samples indicated that nitrification was fairly rapid but that the amount of nitrate or of mineral nitrogen formed was small in comparison with older grassland soils. The soil appeared to become richer in carbohydrate material up to the time of hay cutting and consequently the C/N ratio increased. After this point a steady decrease was observed.

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CXCVI. THE DETERMINATION OF LIGNIN

III. ACID PRETREATMENT AND THE EFFECT OF THE PRESENCE OF NITROGENOUS SUBSTANCES

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DURING the past few years there have appeared a number of papers dealing with the determination of lignin, in most cases, in mature woods. Although there is now a measure of agreement as to the difficulties to be overcome, there is no unanimity as to the most satisfactory procedure which must be adopted. The ideal is obviously to obtain full recovery of all the authentic lignin, without contamination by condensed or polymerized residues from other constituents. Most attention has been given to the interference which may be caused by the presence of certain polysaccharides. Errors from this source may be particularly serious if the time of contact with the 72% sulphuric acid is prolonged, or if the temperature during this digestion is above 20°. It has been suggested that such errors may be minimized by control of the conditions of digestion, or by an appropriate pretreatment designed to remove the interfering substances. A vital point is that the pretreatment should not affect the lignin as a whole or any part of it. Ritter *et al.* [1932] showed that significantly lower lignin figures may be obtained if the material is extracted with boiling water for 3 hr. prior to the addition of strong acid. They did not give any explanation of this effect other than that "extractives" which might be converted into insoluble products are removed. Norman & Jenkins [1934, 1] put forward the case for dilute acid hydrolysis as a pretreatment, in order to remove pentose-containing polysaccharides. They stated that the validity of this pretreatment was not proved beyond doubt, since it is not certain that lignin, as existing in the plant and before coming into contact with 72% sulphuric acid, is completely unaffected by hot dilute acids. The principle of a simple acid pretreatment has been accepted by Phillips & Goss [1936], but recently has been challenged by Bamford & Campbell [1936] on the grounds that the result obtained may still be too high through the formation of insoluble products from partially hydrolysed carbohydrates. It has also been questioned by Cohen & Harris [1937] who take the opposing view that the result after such a treatment is too low owing to the removal of "soluble lignin". Bamford & Campbell [1936] do not reject the principle of an acid hydrolysis, but believe that one long treatment is less satisfactory than several brief treatments, by which means the products of hydrolysis are removed as soon as possible.

The following observations bear on some of the issues raised by the authors mentioned and on the more difficult problem of the disturbance caused by nitrogenous substances.

EXPERIMENTAL

(a) The disturbance caused by non-nitrogenous substances

The presence of pentose or pentose-containing polysaccharides was shown in a previous paper [Norman & Jenkins, 1934, 1] to cause errors in the lignin determination unless special precautions are taken. It was demonstrated that fur-

furaldehyde is slowly produced in contact with 72 % sulphuric acid, and that the cause of the disturbance is the condensation of this aldehyde with lignin and not necessarily the formation of an insoluble condensation product from the furfuraldehyde itself. Lignin in strong sulphuric acid is apparently more reactive than has been supposed, and lignin-aldehyde products have been obtained from all aldehydes tested. The increment in weight of apparent lignin produced varies with the time of contact with the acid but is quite appreciable in as little as 2 hr. (Table I). Furfuraldehyde is more active than formaldehyde, acetaldehyde and

Table I. *Condensation of aldehydes with lignin*

Yield of apparent lignin (ash-free) from 0.8 g. oat straw. 15 ml. 72 % acid for time stated; temp. < 20°, diluted to 600 ml. and boiled 2 hr.

Aldehyde (ml.)	mg. apparent lignin	
	2 hr.	16 hr.
None	138	159
Furfuraldehyde 0.025	154	194
0.05	156	225
0.1	186	294
0.2	214	374
Formaldehyde (40 %) 0.5	176	179
1.0	173	171
Benzaldehyde 0.05	150	168
0.2	152	172
1.0	162	201

benzaldehyde, and in any case itself gives an insoluble condensation product when the combining power of the lignin is satisfied. Aldehydes are not the only compounds to condense with lignin under these conditions; ketones and phenolic substances may also be retained. Even if the lignin has taken up aldehyde to its full ability, phenol may still combine (Table II).

Table II. *Condensation of phenol and formaldehyde with lignin*

Yield of apparent lignin (ash-free) from 1 g. oat straw. 15 ml. 72 % acid for 2 hr.; temp. < 20°, diluted to 600 ml. and boiled 2 hr.

Substance added	mg. apparent lignin
None	178
0.1 ml. 40 % formaldehyde	225
0.2 ml. 40 % formaldehyde	225
0.1 g. phenol	194
0.1 g. phenol + 0.2 ml. formaldehyde	280

Ritter & Barbour [1935] have shown that tannins of the catechol type may cause interference in the lignin determination, presumably through some similar

Table III. *Condensation of tannic acid with lignin*

Yield of apparent lignin (ash-free) from equivalent amounts of water-extracted and acid-hydrolysed wheat straw. Conditions as in Table I

Tannic acid added mg.	mg. apparent lignin from	
	899 mg. water-extracted straw, 2 hr.	623 mg. acid-hydrolysed straw, 16 hr.
None	173	166
50	189	176
100	205	196
200	209	201

condensation. Tannic acid also produces substantial increases in apparent lignin (Table III).

These observations make it probable that any phenolic substance present in the plant material, or any aldehydic substance produced by the action of the concentrated sulphuric acid, may condense with the lignin and give an erroneously high result. The substance in question may not itself give an insoluble residue with 72 % acid, and the absence of such a residue under any chosen conditions of digestion is not conclusive evidence that no interference is caused if lignin is present. For example Bamford & Campbell [1936] sought conditions of digestion that would give minimum yields of lignin and recommended 5-6 hr. at 10°. Xylose treated under these conditions gave only 0.07 % of insoluble residue. The experiment should however have been conducted in the presence of lignin, since it is quite possible that soluble products capable of condensing with the lignin might be formed. If this is done it is by no means so certain that the effect of the pentose is negligible, though the results were not consistent when a variety of materials was examined.

Table IV. *Effect of addition of xylose to lignin determination*

Yield of apparent lignin % from alcohol-benzene-extracted materials. 0.75 g. xylose added to 0.75 g. material + 15 ml. 72 % sulphuric acid. Digestion 6 hr. at 10°, diluted to 600 ml. and boiled 2 hr.

Material	Alone	With xylose
Oak	26.2	26.9
Hydrolysed oak*	32.2	33.2
Silver fir	31.1	32.0
Hydrolysed silver fir	36.9	38.2
Hay	14.7	16.2
Hydrolysed hay	27.0	26.2
Wheat straw	17.9	17.7
Hydrolysed wheat straw	25.3	25.4
Jute	12.2	12.2
Pine	26.8	27.5

* All hydrolyses were effected with 5% H_2SO_4 for 1 hr. The hydrolysed samples are not expressed on original basis.

In four cases out of six the addition of xylose to an unhydrolysed material resulted in an increase in apparent lignin. The increments are not large considering the high proportion of pentose added. A comparison of the hydrolysed with the unhydrolysed materials does not suggest that the pentose-containing polysaccharides of the latter are causing any serious error in the determination, though it cannot be said with confidence that they are wholly without effect. Conditions which result in complete solution of the cellulose without at the same time causing breakdown of pentoses may be impossible to find, so that the choice may have to be made of conditions most nearly approximating to that ideal.

(b) *Acid hydrolysis as a pretreatment*

The use of a single dilute acid hydrolysis as a pretreatment to the determination of lignin has been criticized by Bamford & Campbell [1936] as ineffective, because with some abnormal woods results higher than on the untreated material were subsequently obtained. They say, however, that "greater accuracy may conceivably be introduced... provided that the products of hydrolysis are removed as soon as possible" and suggest that several brief acid treatments may be more satisfactory. A very wide range of plant materials has been examined

in this laboratory during the past 3 years, and in no single case has the lignin figure obtained after hydrolysis been higher than that on the untreated material. In the aberrant woods mentioned by Bamford & Campbell [1936] the pentose-containing substances seem to have been peculiarly resistant to hydrolysis. If the acid hydrolysis were intended to prevent any possible pentose disturbance, as was presumably the case, then the treatment was futile, and a lower lignin figure could hardly have been expected. The fact that slightly higher figures were recorded in some cases may have been due either to the formation of some non-lignin insoluble product, as suggested by Campbell, or to the condensation of some soluble product with the lignin or possibly to both. The former is to be deduced from the deposition of an insoluble residue from the filtered acid extract on further boiling as recorded by Bamford & Campbell [1936] or on standing for some days [Cohen & Harris, 1937] as has also been observed here. The second possibility has been demonstrated by the addition of aldehydes to the acid used for hydrolysis (Table V).

Table V. *Effect of addition of aldehydes to the acid hydrolysis*

Yield of apparent lignin from 1 g. straw after hydrolysis with 5% sulphuric acid.

Digestion conditions as in Table I

Aldehyde added	mg. apparent lignin
None	150
1 ml. furfuraldehyde	159
2 ml. furfuraldehyde	175
2 ml. 40% formaldehyde	157

The statement that slightly lower yields of lignin are obtained by successive brief treatments with acid instead of one longer treatment has been confirmed on some materials. The important proviso must be added that the total material removed by the brief treatments must be roughly equal to that extracted by continuous hydrolysis. After four extractions with 100 ml. 5% sulphuric acid for successive periods of 5, 10, 15 and 20 min., the lignin yields from oat straw and oak were lower than if one continuous hydrolysis were given (Table VI).

Table VI. *Effect of discontinuous acid hydrolysis on apparent lignin content*

mg. of apparent lignin from 1 g. alcohol-benzene-extracted material. All results expressed on original materials. Digestion conditions as in Table I for times stated

Treatment	Oat straw				Oak			
	Residue	Furfur- aldehyde yield	2 hr digestion	16 hr. digestion	Residue	Furfur- aldehyde yield	2 hr. digestion	16 hr. digestion
1. None	100	15.6	17.4	19.4	100	14.4	23.7	26.9
2. 100 ml. 1% acid for 2 min. 5 times	85.0	14.8	16.0	18.3	86.1	14.1	20.2	22.8
3. 100 ml. 5% acid for 2 min. 5 times	79.5	12.8	15.6	16.6	83.7	12.1	20.6	22.5
4. 100 ml. 5% acid for 5, 10, 15 and 20 min.	62.9	5.8	14.5	14.6	73.4	7.1	20.0	20.7
5. 100 ml. 5% acid for 1 hr.	61.9	4.5	15.8	15.9	70.1	6.3	20.4	21.1

If the difference between continuous and intermittent hydrolysis is due to one or both of the possibilities mentioned above, the effect should be enhanced by using the acid extract of one portion to hydrolyse another portion. Amounts of 3 g. were treated for 5 min. with 100 ml. 5% sulphuric acid and filtered, the extract then being employed to hydrolyse an equal portion of the same material for a period of 1 hr. Lignin determinations were carried out on portions of the residues, the lignin yield in most cases being found to be appreciably higher than if a simple or intermittent hydrolysis were given.

Table VII. *Effect of boiling with previous acid extract*

Treatment	Details as Table VI								
	Wheat straw			Wheat plants			Pine		
	Residue	2 hr. digestion	16 hr. digestion	Residue	2 hr. digestion	16 hr. digestion	Residue	2 hr. digestion	16 hr. digestion
1. 5% acid for 1 hr.	62.8	15.3	15.5	50.1	13.1	13.1	80.8	27.1	27.5
2. Boiled for 1 hr. with 5% acid extract 5 min.	63.4	16.0	16.5	51.9	13.6	13.5	81.2	27.1	27.6
3. 5% acid for 5 min. then 55 min.	62.0	14.8	14.8	50.8	13.0	13.4	80.6	26.8	27.3
4. 5% acid for 5 min. then 10 and 45 min.	61.1	14.0	14.6	51.4	12.4	13.0	82.4	26.7	27.6

The filtered acid extract of most materials on standing for days or weeks deposits a brownish black precipitate, instantaneously soluble in alkali to give a deep-coloured solution. Bamford & Campbell [1936] mention the formation of a similar precipitate on continued boiling of an acid extract and presumably consider it to be formed from the hydrolysed carbohydrates. Cohen & Harris [1937] prefer to think that a portion of the lignin of woods is soluble in dilute acid or even in hot water and that the flocculent precipitate which separates out from acid extracts on standing is a polymerized insoluble form of this soluble lignin.

They state that this precipitate from an acid extract of maple wood had a methoxyl content precisely similar to that of the true lignin residues, and moreover gave the characteristic colour changes on chlorination and addition of sulphite. If this view is correct the use of any acid hydrolysis treatment is to be avoided except as a means of distinguishing between "soluble" and "insoluble" lignin. Their results suggest that the cleavage between the two may be clear cut, since prolonged acid hydrolysis did not give lower lignin figures than a 2 hr. treatment. The conception of a separate water-soluble form of lignin is novel and will require to be substantiated by preparation and characterization. Its existence would provide an explanation for the considerable differences in apparent lignin yield under the same digestion conditions when an aqueous extraction is employed as pretreatment, as in the Ritter-Seborg-Mitchell method, and "soluble" lignin would take the place of the interfering "extractives" postulated by them. At the moment the evidence for its existence is not extensive and depends largely on a colour test which may not be as specific as has been supposed. It is not necessary to carry out a prolonged aqueous treatment to obtain an extract which will give a red colour with sulphite after chlorination. Wood or straw treated with warm water for 2 or 3 min. will give an extract with this property. If excess of baryta is added to the filtered aqueous extract of mature straw, a bulky olive-brown precipitate is obtained which settles

readily. This may be washed by decantation with water containing baryta and finally with boiling water. The dried product suspended in water gives a red colour on addition of sulphite after chlorination, and might be said on these grounds to contain lignin. Acid permanganate is rapidly decolorized, and with ferric chloride a brown-purple colour is obtained. The product contains about 30 % Ba. If treated with 72 % sulphuric acid a light brown insoluble residue also giving the lignin colour test is obtained. The yield of ash-free residue in 16 hr. was about 30 %, when expressed on the Ba-free product. This crude product has not yet been fractionated but must be considered to contain either lignin, some lignin-former or precursor, or some substance, probably phenolic, giving positive lignin tests and forming an insoluble residue with strong sulphuric acid.

In view of the results obtained by Cohen & Harris [1937] and the observations above, the water-soluble and acid-soluble fractions of woods and straws would repay further investigation. The information available at present does not seem to warrant the immediate rejection of acid hydrolysis as a pretreatment in the lignin determination, although doubts as to its suitability are certainly raised.

(c) *The effect of the presence of nitrogenous substances*

The lignin residues from many plant materials containing protein may retain appreciable amounts of nitrogen combined with the lignin. The nitrogen is not present as protein, but probably as protein degradation products of variable size. The magnitude of the error thus caused varies with the conditions of digestion and is generally more or less proportional to the protein initially present [Norman & Jenkins, 1934, 2]. So far no means of avoiding this disturbance has yet been found; most expedients which have been tried have resulted also in an effect on the lignin itself. Hydrolytic pretreatment with dilute acid is partially successful in minimizing the disturbance and has been employed in work on agricultural materials containing proteins. The increments in apparent lignin caused by the addition of a known amount of protein tend to vary inversely with the digestion time and are not reduced by digestion at low temperatures.

In an attempt to obtain further information as to the nature of the disturbance, the effects of addition of a number of nitrogenous substances of varied type were determined (Table VIII) following two procedures. In the first the Ritter-Seborg-Mitchell method, which involves hot aqueous extraction for 3 hr. and subsequent digestion with 72 % acid for 2 hr., was adopted, and in the second the material was hydrolysed with 5 % acid for 1 hr. prior to 16 hr. digestion. Equivalent amounts of the extracted residues were taken and the nitrogenous substance mixed in before the addition of the 72 % acid. Considerable increments in apparent lignin were produced by the proteins and also by fungus chitin, the nitrogenous group of which is in the form of hexosamine. The ratios of increment in lignin over increase in nitrogen were variable and in nearly all cases over 10. The addition of simple amino-acids did not result in any large increase in apparent lignin except in the case of tyrosine in which the effect was probably due in part to the condensation of the phenolic residue with the lignin. The water-extracted straw having 2 hr. digestion seemed more affected than the acid-extracted material with a longer digestion period, as has been observed before. This was particularly the case with the more complex nitrogenous substances such as creatinine, guanine and nucleic acid. The general conclusion to be drawn from Table VIII is that the disturbance caused by proteins must be

Table VIII. *Effect of addition of nitrogenous substances*

Apparent lignin from 1 g. wheat straw (=0.899 g. water-extracted or 0.623 g. acid-extracted straw).

Substance added (mg.)	Water-extracted straw, 2 hr. digestion		Acid-extracted straw, 16 hr. digestion	
	Apparent lignin mg.	mg. N in lignin	Apparent lignin mg.	mg. N in lignin
None	173	1.7	166	1.3
Casein	50 203	4.7	190	3.8
"	100 214	7.0	201	6.0
Wheat gluten	50 207	4.5	186	3.3
"	100 240	5.8	204	5.1
Fungus chitin	50 184	2.0	168	1.5
"	100 187	2.0	165	1.8
"	250 192	2.9	178	2.5
"	500 205	3.8	192	3.3
Glycine	50 177	1.8	166	1.3
"	100 178	1.6	166	1.3
"	200 176	1.9	170	1.3
Alanine	50 185	1.7	164	1.4
"	100 190	1.8	166	1.4
"	200 182	1.7	162	1.3
Glutamic acid	50 170	1.5	168	1.3
"	100 174	1.5	169	1.5
Aspartic acid	50 173	1.7	170	1.5
Asparagine	50 178	1.6	166	1.4
"	100 177	1.7	167	1.6
Tyrosine	50 191	2.4	172	2.2
"	100 190	2.6	169	2.4
Acetamide	50 172	1.7	166	1.2
"	100 179	1.5	166	1.3
Biuret	100 176	1.7	162	1.9
Betaine-HCl	50 170	1.6	164	1.5
"	100 173	1.7	168	1.3
Creatinine	50 179	1.8	168	1.4
"	100 184	2.1	166	1.4
Guanino-HCl	50 177	1.7	164	1.2
"	100 183	1.9	167	1.3
Yeast nucleic acid	50 180	1.8	171	1.4
"	100 195	1.9	172	1.4

due to the linkage of large fission products, not necessarily through the amino-group. In many cases these fragments which become attached must have suffered partial deamination, since the nitrogen content of the increment produced is often, though not always, far above 6%. The expedient which must ultimately be adopted may have to be directed more towards the breakdown of the protein molecule into small units, rather than to the removal of amino-groups.

SUMMARY

1. Various aspects of the determination of lignin in plant materials are discussed. Interference is shown to be produced by the condensation of aldehydes or phenolic substances with lignin. Proof of the absence of pentose disturbance under any particular conditions must be given by experiments carried out in the presence of lignin.

2. The observation that after intermittent acid hydrolysis slightly lower yields of lignin are obtained than after continuous hydrolysis is confirmed. This may be due either to the formation of some non-lignin insoluble product, or to the condensation of some soluble product with the lignin.

3. The enhanced yields of apparent lignin caused by the presence of protein must be caused by the condensation of large protein fragments, partially deaminated. The disturbance caused by simple amino-acids and nitrogenous bases is of a much lower order.

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CXCVIII. THE HEMICELLOSES

III. EXTRACTION AND PREPARATION

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OF the chief cell wall substances, the hemicelluloses are probably the most difficult constituents to prepare in a satisfactory state of purity. The conditions of extraction recommended by various workers differ considerably, and the criteria of purity applied are not uniform. The observations in this paper bear on various aspects of the extraction and purification of this group of polysaccharides.

(a) Pretreatment with alcoholic sodium hydroxide

The extraction of hemicelluloses is carried out with cold or hot solutions of NaOH, in which lignin is also soluble to a considerable extent. In order to reduce the amount of lignin passing with the main extract Norris & Preece [1930] recommended a preliminary delignifying treatment with alcoholic NaOH, and this procedure has in fact been employed subsequently in a number of investigations. In an earlier paper of this series [Norman, 1935] evidence was given to show that this reagent when hot has a degradative effect on the polyuronide hemicelluloses and to some extent also on the xylan associated with the cellulose so that a considerable loss of furfuraldehyde-yielding constituents takes place and there is some chance of degradative change in those remaining. Different materials were found to vary in degree of susceptibility to this reagent, but in nearly all cases there was no doubt that as a result of the pretreatment the yield of hemicellulose would be reduced. In recent investigations of the hemicelluloses of hop and of maize cobs Angell & Norris [1936] have claimed that the alcoholic NaOH treatment did not result in any loss of hemicellulose from these materials on the grounds that the precipitate obtained on acidification of this extract failed to yield any furfuraldehyde. However, it might be pointed out that this evidence alone is not conclusive, since the degradative effect of this reagent, if any, is not necessarily similar to acid hydrolysis, and the fission products formed would not necessarily yield furfuraldehyde or even be precipitated by simple acidification. To obtain conclusive proof it must be established by analysis that the residual material after extraction is not appreciably lower

Table I. *Extraction of wheat straw with 1% NaOH in 50% alcohol*

2.5 g. portions boiled with 100 ml. for 1½ hr. Results expressed as % of original material

	Residue	Cellulose	Furfur- aldehyde from cellulose	Xylan in cellulose	Total furfur- aldehyde	Furfur- aldehyde from polyuronides	Lignin
Untreated	—	53.5	10.35	16.1	16.52	6.2	15.1
Extracted	64.95	51.5	8.62	13.4	13.06	4.4	5.0

in furfuraldehyde constituents than before. Examples in which this was not the case are to be found in the previous paper [Norman, 1935] and an additional experiment is given in Table I.

The analyses of the residue show conclusively that with this material pre-treatment with alcoholic NaOH, although effective in delignification, did result in a considerable loss of both types of furfuraldehyde-yielding constituents. The degradative attack was such that only a part of the furfuraldehyde could be recovered from the extracts and none from the precipitate obtained on simple acidification (Table II). The straw had lost constituents yielding 3.7% furfuraldehyde, of which only about one-quarter could be found in the extract. The

Table II. *Experiments on alcoholic NaOH extracts from wheat straw*

All in duplicate. Results expressed as % of original straw		%
A. Extract neutralized, concentrated under reduced pressure to remove alcohol. Yield of furfuraldehyde	0.84
B. Extract acidified with excess HCl and filtered. Weight of precipitate	...	1.37
C. Filtrate from B neutralized and concentrated under reduced pressure to remove alcohol. Yield of furfuraldehyde	0.92
D. Extract neutralized, concentrated under reduced pressure to small volume, half volume concentrated HCl added. Weight of precipitate	...	11.89
E. Filtrate from D. Yield of furfuraldehyde	0.53
F. Extract brought to pH 4.0 and filtered. Weight of precipitate	...	3.06
G. Filtrate from F neutralized and concentrated under reduced pressure to remove alcohol. Yield of furfuraldehyde	0.88

products produced have not been investigated since they are of no special moment. Both volatile and non-volatile acids have been found in the extract in amounts significantly greater than are obtainable from the original material by simple dilute alkali treatment. 4.8% volatile acid calculated as acetic was obtained from the wheat straw extract and non-volatile acid with a titration figure of approximately half that of the volatile.

From these experiments the conclusion must be drawn that the only satisfactory proof of the absence or magnitude of the degradative attack caused by hot alcoholic NaOH on the furfuraldehyde-yielding constituents is to be obtained by comparison of the analyses of the material before and after extraction, and not by examination of the extract.

(b) *Conditions of extraction of hemicelluloses*

Hemicellulose preparations are obtained from alkaline extracts of the material under investigation, but the precise conditions employed are variable. The strength of the alkali is not usually less than 4%. Some workers confine themselves to exhaustive cold extraction, whilst others follow a cold extraction with one at the boil. There is now a considerable weight of evidence pointing to some form of association between the polyuronide hemicelluloses and lignin, which probably amounts to actual linkage [Harris *et al.* 1934; Norman & Shrikhande, 1935]. If the existence of such a complex be recognized, preparatory work may be simpler, in that the purpose of the treatment given must be to rupture the association. This may presumably be effected with alkali, which simultaneously dissolves the hemicellulose and much of the lignin, or more easily by chlorination or oxidation of the lignin. Simple alkali extraction in the cold even if repeated many times does not usually result in complete removal of

the polyuronide hemicelluloses from mature lignified tissues such as woods. Sands & Nutter [1935] showed that if exhaustively extracted wood is then delignified by alternate treatments with chlorine water and 10% ammonium hydroxide, subsequent extraction with cold alkali will remove most of the residual hemicelluloses. Their observations have been confirmed, but their procedure is not recommended for all purposes because there is undoubtedly some loss of hemicellulose in the ammonia extracts. They also employed 10% NaOH for the final extraction, a concentration which is unnecessarily high and causes too great an attack on the β - and γ -cellulose fractions. However, their procedure in principle depends on the liberation of the residual hemicellulose by attacking and removing the lignin. The purity of the final preparation is not much improved by the solution of the lignin and oxidized lignin in ammonia, because the initial NaOH extract of most materials contains a considerable quantity of lignin. A procedure which gives an extensive removal of hemicellulose is to chlorinate the residue obtained from the initial cold alkali treatment and after washing with a little water immediately to extract again with cold alkali of the same strength. Prior to the initial treatment the material should be extracted if necessary with organic solvents, with warm water and with dilute ammonium oxalate if pectin is present. Analyses of some materials which received one treatment with 4% NaOH for 24 hr. and also a second treatment after chlorination in faintly acid solution are given in Table III.

Table III. *Extraction of hemicelluloses by cold 4% NaOH before and after chlorination*

Initial treatment 24 hr., chlorination 15 min., final treatment 24 hr.
All results expressed as % of original material

Material	*	Residue	Lignin	Cellulose	Furfur-aldehyde from cellulose	Total furfur-aldehyde	Polyuronide furfur-aldehyde
Barley straw	1	—	16.4	48.6	7.4	18.7	11.3
	2	58.6	7.5	43.9	5.0	7.0	2.0
	3	39.7	3.3	35.2	2.5	3.4	0.9
Wheat plants	1	—	12.6	38.4	6.5	12.1	5.6
	2	48.9	5.9	35.8	4.9	6.4	1.5
	3	33.6	2.9	28.2	3.0	4.1	1.1
Oak	1	—	22.3	53.9	9.2	13.4	4.2
	2	83.9	18.8	52.9	8.1	10.6	2.5
	3	75.0	15.6	51.4	7.3	8.6	1.3
Wheat straw	1	—	16.6	56.7	7.7	16.6	8.9
	2	60.8	7.3	46.0	5.8	8.9	3.1
	3	49.6	4.2	44.5	4.6	6.0	1.4
Jute	1	—	11.7	74.9	7.3	10.6	3.3
	2	86.0	10.9	69.7	5.1	7.2	2.1
	3	75.9	6.9	67.6	4.1	5.1	1.0

* 1 = Untreated. 2 = Initial alkali treatment only. 3 = Residue from 2 chlorinated and re-extracted with alkali.

It will be seen that these two treatments taking only 48 hr. have brought most of the polyuronide hemicelluloses into solution. More can be obtained by a further treatment, but this is attended by other dangers, as will be mentioned later.

The possibility of using much more dilute alkaline solutions and carrying out the extraction at the boiling point has been examined. The assumption that hemicelluloses are unaffected by hot alkali seems generally to be made, though

without absolute proof, but it is unlikely that solutions as dilute as were employed would result in degradation. The procedure adopted was to boil for 30 min. and to alternate this treatment with brief chlorination (Table IV).

Table IV. *Extraction of hemicelluloses from oat straw by dilute alkaline solution*

Boiled for 30 min., chlorinated for 10 min.

Treatment	Residue	Lignin	Cellulose	Furfur- aldehyde from cellulose	Total furfur- aldehyde	Polyuronide furfur- aldehyde
Untreated	—	15.9	52.9	9.1	15.3	6.2
0.1% NaOH	79.6	12.3	52.0	8.2	14.1	5.9
+ 1 chlorination	72.5	8.9	50.5	8.0	13.3	5.3
+ 2 chlorinations	68.4	6.6	50.1	7.8	12.9	5.1
0.5% NaOH	58.5	4.4	50.7	8.5	10.2	1.7
+ 1 chlorination	51.4	1.9	45.4	7.5	8.8	1.3
+ 2 chlorinations	47.7	0.7	43.5	6.2	7.5	1.3
0.5% Na ₂ CO ₃	77.1	11.6	52.8	8.7	13.8	5.1
+ 1 chlorination	67.3	7.6	51.0	8.0	13.1	5.1
+ 2 chlorinations	60.2	4.1	46.7	7.7	10.8	3.1
2% Na ₂ CO ₃	70.3	8.4	52.1	8.7	12.6	3.9
+ 1 chlorination	60.5	4.3	48.3	7.3	10.8	3.5
+ 2 chlorinations	53.6	1.9	48.1	6.9	8.9	2.0
M/5 K ₂ HPO ₄	82.4	12.8	51.5	8.7	14.6	5.9
+ 1 chlorination	78.0	11.0	51.0	8.8	14.5	5.7
+ 2 chlorinations	73.5	8.9	50.8	8.3	14.0	5.7

The results indicate that removal of hemicelluloses can be achieved under conditions considerably less drastic than sometimes employed. No general method can be suggested because different materials vary considerably in behaviour to these more dilute solutions, but a procedure using first 2% Na₂CO₃ and then, after chlorination, 0.5% NaOH would result in the solution of the major part of the hemicelluloses.

(c) *Purity of hemicellulose extracts and preparations*

Cold alkali, in concentrations from 4 to 10%, as commonly employed, extracts hemicelluloses of two types, as well as lignin. Not only do the encrusting polyuronide hemicelluloses go into solution but also some of the cellulosan associated with the cellulose. This dual source of the hemicelluloses has not been fully recognized and it is a strong criticism of fractionation methods that no clear separation is effected. In most cases therefore the preparations obtained have been mixtures of polyuronide hemicelluloses with non-uronic material from the cellulose. This point is discussed more fully elsewhere [Norman, 1937]. Up to a point the polyuronide hemicelluloses are more easily extracted than the cellulosan and accompanying material from the cellulose, but as the conditions of extraction become more drastic so does the proportion of the latter to the former increase. In most materials, other than softwoods, the cellulosan is xylan, and an investigation of the fractionation of an alkaline extract of isolated cellulose has showed that the major part of the xylan is precipitated in the B₁ fraction of the system developed by Norris & Preece [1930]. The hexosan simultaneously removed is not precipitated by copper and should therefore be found in the B₂ or C₂ fraction.

The improved fractionation procedure described by Angell & Norris [1936] may make it possible to distinguish more satisfactorily between the polyuronide hemicelluloses and polysaccharides having their origin in the cellulose, both of which will always be present in a simple alkaline extract if the concentration is high. Some idea of the relative proportions may be obtained from Table V in

Table V. *Composition of residues from hemicellulose extraction*

Extracted twice for 24 hr. with cold 4% NaOH, chlorinated 15 min., and re-extracted for 24 hr. with cold alkali. All results expressed as % of original material

Material	Residue	Lignin	Cellulose	Furfur- aldehyde from cellulose	Total furfur- aldehyde	Poly- uronide furfur- aldehyde	P/T*
Oat straw	—	15.9	52.9	9.1	15.3	6.2	
Residue	31.5	2.7	26.8	2.7	3.5	0.8	46
Holly wood	—	19.0	53.7	7.3	14.6	7.3	
Residue	60.6	12.1	47.5	4.0	4.9	0.9	66
Wheat straw R	—	16.0	53.5	7.7	16.4	8.7	
Residue	51.5	4.9	43.1	4.9	6.6	1.7	71
Wheat straw M	—	15.1	53.5	10.3	16.5	6.2	
Residue	41.4	2.1	38.3	3.7	3.8	0.1	48
Wheat straw S	—	15.1	54.3	9.0	16.6	7.6	
Residue	41.0	1.3	39.4	3.6	4.2	0.6	56
Wheat plants	—	12.6	38.4	6.5	12.1	5.6	
Residue	28.3	—	27.0	2.4	2.9	0.5	55

* P/T - $100 \times \text{Polyuronide furfuraldehyde removed} / \text{Total furfuraldehyde removed}$.

which are given the analyses of the residues after exhaustive extraction with 4% NaOH in the cold as commonly employed, followed by brief chlorination and further treatment with alkali of the same strength. The ratio P/T in the final column is an expression in terms of furfuraldehyde of the contribution made by the polyuronide hemicelluloses to the total furfuraldehyde in the extract. The proportions of polyuronide hemicellulose to cellulose-derived material are of course not the same as this ratio, but the figures are sufficient to show that in most hemicellulose preparations there is a substantial contribution from the cellulose. This is equally apparent if a similar examination be made of the analyses given in Table I. Crude or unfractionated hemicellulose preparations were obtained from each of the combined extracts from holly wood, wheat straw M and wheat straw S by precipitation with acid and excess alcohol. After purification the furfuraldehyde yield of the preparations was determined, and found to be respectively 55.1, 52.5 and 51.2% on the ash- and lignin-free basis. From the data in Table V it is then possible to calculate approximately the amount of cellulose-derived xylan and hexosan present, assuming no losses. In these three preparations this amounted to:

	Holly wood %	Wheat straw M %	Wheat straw S %
Xylan	29.5	42.7	34.6
Hexosan	5.7	19.7	26.5

Too much weight cannot be attached to these figures since the experimental errors are magnified considerably, but they serve to emphasize again the fact that the alkali extract of materials such as straw may not consist predominantly of polyuronide hemicellulose. If it is desired to investigate this fraction, and if fractionation procedures do not effect a good separation, as seems to be the case

at present, other means of extraction will have to be sought. There are indications in Table IV that a brief hot extraction with 0.5% alkali has a much less drastic effect on the cellulose than extraction with cold 4% solutions, and if purity were considered more important than the completeness of extraction, such a treatment for 30 min. or less would be far better. Alternatively there is the possibility of working up the sulphite liquors obtained in the isolation of cellulose, though there might be difficulties in getting the preparations entirely free from lignin sulphonic acids. Whatever means of extraction is adopted an analysis of the residue is desirable in order that the relative proportions of the various constituents removed may be known.

There is another aspect of the purity of hemicellulose preparations which is sometimes overlooked. Extraction of any plant material with alkali removes lignin, often to a considerable extent, some of which may later be precipitated according to the conditions adopted. If the hemicellulose is carefully purified by redissolving in alcohol and precipitating with acid or acid alcohol a number of times and is thoroughly washed with alcohol, the lignin remaining in the final preparation is rarely more than 2-3%. To reduce this further is often difficult. Crude preparations may contain as much as 10-15%. Very few investigators have determined the lignin contents of their preparations. Some indeed have considered the insoluble residue remaining after simple acid hydrolysis to be an integral part of the hemicellulose molecule. For example, Sands & Gary [1933] reported 6.5% of "body X" in the hemicellulose A fraction from mesquite wood. Because of the strong probability of its presence this must be assumed to have been lignin until the opposite is proved, although there is the further possibility of formation of a small amount of insoluble matter as a result of the action of acid on pentose groups.

In order to minimize the amount of lignin to be found in the final preparation experiments have been directed along two lines: (1) the reduction of the amount of lignin in the original alkaline extract before precipitation of the hemicellulose, (2) the reduction of the amount of lignin still retained by the hemicellulose preparation precipitated in the usual manner. Of these two alternatives the latter is the more effective, since the former cannot be achieved completely without some loss of hemicellulose. The procedure which has been found most effective is to redissolve the precipitated hemicellulose in dilute alkali, either before or after fractionation and to add a small amount of dilute sodium hypochlorite followed by sufficient acetic or hydrochloric acid to liberate the chlorine. After standing 5-10 min., the minimum quantity of alcohol to effect full precipitation is added together with more acid if necessary. The precipitate is immediately filtered off and washed carefully with more alcohol of the same concentration. It is of importance that the alcohol concentration should not be higher than necessary since the lignin oxidation products seem to be less soluble in high concentrations. In practice two such treatments have been found to give excellent preparations. The expedient of first precipitating all the hemicelluloses from the extract by addition of acid and excess alcohol has been adopted followed by fractionation of the partially purified product. One chlorination may conveniently be given at this stage and the treatment repeated later if necessary on the individual fractions after separation. Preparations of low ash content have been obtained more readily by using hydrochloric acid for acidification rather than acetic acid as commonly employed.

SUMMARY

1. If hot alcoholic NaOH is to be used as a pretreatment before hemicellulose extraction, it should be established by analysis of the residue that the furfuraldehyde-yielding constituents have not been attacked. The fact that no furfuraldehyde can be obtained from the precipitate formed on acidification of the alcoholic extract is not conclusive proof that no degradative attack has occurred.

2. Extensive removal of hemicellulose material may be effected by extraction with cold 4 % NaOH alternated with brief chlorinations. Such extracts may contain a high proportion of cellulose-derived polysaccharides. Brief extraction with hot, more dilute alkali has a less drastic effect on the cellulose and such extracts may consist largely of polyuronide hemicelluloses.

3. The lignin content of hemicellulose preparations should always be determined. It may be reduced by brief treatment with chlorine and thorough washing with alcohol of moderate concentration.

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CCXC. THE ASSOCIATION OF XYLAN
WITH CELLULOSE IN CERTAIN
STRUCTURAL CELLULOSES.

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(Received 30 September 1936.)

THE cellulose of most plant materials and woods differs from cotton cellulose in many respects. In the first place, separation from other cell wall constituents can only be achieved by a more or less severe treatment, in contrast to that of the cotton hair which can readily be purified. In the second place, the product obtained, while consisting mainly of "true cellulose" as typified by that of cotton, is not exclusively so, and contains also other polysaccharides, intimately associated and tenaciously retained. For this group Hawley & Norman [1932] have suggested the name "cellulosan" implying thereby a hexosan or pentosan found with the cellulose and held in some way by it. In this paper will be described some observations on the celluloses of certain plants, mostly cereal straws, which have a high cellulosan content. Attempts have been made by chemical means to obtain information as to the relationship between the "true" cellulose and associated cellulosans. A parallel investigation, involving the X-ray examination of cellulose fibres high in cellulosan, has been carried out in conjunction with Mr W. T. Astbury and will be reported later.

The present concept of the structure of the cellulose molecule has been developed by a combination of chemical and physical methods, which have shown that the peculiar and valuable properties of cellulose are not conferred by its molecular size so much as by the state of molecular aggregation in which it ordinarily exists. While actual replacement of any of the side hydroxyls in the units of the cellulose chain would not affect the strength of the chain longitudinally, the increased separation of the chains by the substitution of larger groups for the hydroxyls would tend to weaken the fibrous structure of the aggregate by overcoming the secondary valency forces responsible for lateral stability. It is stated that nitrocellulose retains its fibrous structure better than the acetate, since in the latter wider separation is brought about. In view of these facts, the existence of lignocellulose or pectocellulose as a definite combination between oriented cellulose and lignin or pectin cannot be admitted and the possibility of direct combination between cellulose and cellulosans is similarly ruled out. The evidence to be presented is in accord with the view that the cellulosan molecules participate in the cellulose micellae and crystallites in the same way as do the individual cellulose chains. The commonest cellulosan is xylan, though in *Gymnosperms* mannan is characteristic. The xylan from *esparto* has been shown to be a 1:4-anhydroxylose, the xylose units being of the normal amylen oxide type [Hampton *et al.*, 1929]. A terminal group of *l*-arabinose has also been detected in this particular case [Haworth *et al.* 1934]. The xylan unit therefore differs from the glucose unit in cellulose only in that the terminal carbinol group is missing. Such units are of the same size and would occupy the same space

longitudinally. The mannan formed in the cellulose from Gymnosperms has not been investigated, but by analogy is likely to be also a 1:4-anhydrohexose and, therefore, sterically similar in size to the cellulose and xylan with which it is so intimately associated.

The cellulosans are probably laid down with the cellulose as it is formed and participate in the oriented bundles of molecules that form the micellae of the fibres. They would be retained by secondary valency forces just as the cellulose chains are themselves stabilized in that way. Because of the absence of a projecting carbinol group, in the case of xylan at least, these forces might be expected to be less powerful than those between the cellulose molecules themselves. Some evidence will be presented later on the relative rates of removal of xylan and mannan from natural celluloses containing both. Further, the molecular size, or rather the length of the chain of sugar units, is considerably less in the case of the cellulosans than in cellulose itself. As a result of their retention by secondary valencies weaker than those between the cellulose chains and of their smaller molecular size they might be expected to be extractable, if with some difficulty. The actual form of participation of the cellulosans in the micellae is, at present, a matter of speculation. They undoubtedly form a normal integral part of the cellulosic structure of the plant cell wall and fibres, and any study of natural celluloses should include this group in as unchanged a condition as possible.

PREPARATION OF CELLULOSE.

Norman & Jenkins [1933] described a method for the determination of cellulose which could readily be adapted for large-scale preparations. Previously chlorinations for the removal of lignin have been carried out by some modification of the original procedure of Cross & Bevan [1918] employing gaseous chlorine, by which means it is impossible on a laboratory scale to treat more than a few g. at a time. By the use of dilute hypochlorite solutions quantities up to 1 kg. may be readily treated. Six or seven treatments are required for such materials as cereal straws in bulk and rather more for woods. The final washing must be continued until no trace of sulphite is detectable in the filtrate.

A. EFFECT OF HEAT ON CELLULOSE PREPARATIONS.

Cellulose preparations containing cellulosan undergo an irreversible change on oven-drying, as a result of which a fraction becomes soluble in hot water. If this be removed and the preparation again oven-dried, a further but smaller fraction can be extracted. This process can be repeated apparently indefinitely. Fig. 1 shows the losses from a sample of oven-dried wheat straw cellulose on boiling with water, the cellulose being oven-dried overnight between each

Table I. *Extraction of wheat straw cellulose, wet and oven-dried.*

Treatment	Total loss %		Loss of xylan %	
	Wet	Dry	Wet	Dry
3.0% Na_2SO_3 2 x 20 min. hot	5.0	7.6	1.1	2.8
0.25% NaOH 1 hour hot	11.7	19.0	3.3	6.0
1.0% NaOH 1 hour hot	17.5	22.0	5.4	7.2
4.0% NaOH 3 hours cold	18.8	22.5	13.2	16.4
0.25% H_2SO_4 1 hour hot	9.4	11.7	4.7	6.2
2.5% H_2SO_4 1 hour hot	11.9	15.6	7.7	10.4

Initial furfuraldehyde yield 13.88%, equivalent to 21.59% xylan.

treatment. After the fifth extraction the xylan content of the residue was determined. Whereas the aggregate loss was 18.8% the xylan removed accounted for only 8.9%, or about half.

The effect, therefore, is not solely concerned with the cellulosan fraction. Further, the effect of heat-drying also renders the cellulose preparations more susceptible to extracting and hydrolysing agents, as shown in Table I in which the losses from wet and dried preparations are compared. Again, the differences are not wholly due to an effect on the xylan, and must arise in part from a change in properties of a portion of the "true" cellulose fraction.

To determine whether this effect is due to the removal of water in drying or the application of heat, or both, a large batch of oat straw cellulose was subdivided and dried in different ways. These samples were then extracted by

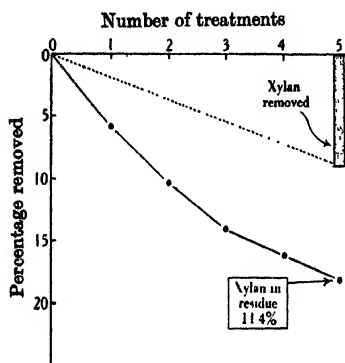


Fig. 1. Water-soluble material produced on repeated drying of wheat straw cellulose (xylan 20.3 %).

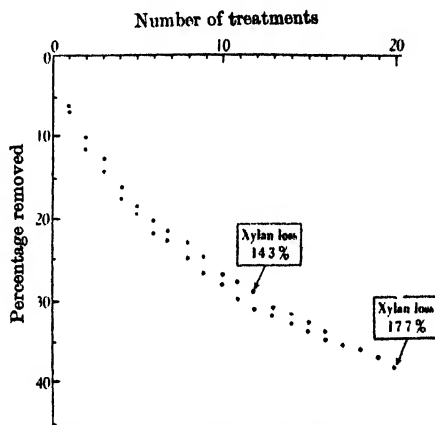


Fig. 2. Repeated extraction and drying of oat straw celluloses.

boiling with water for 15 min., and since the effect of certain initial treatments was noticed to persist after the first extraction, all the residues after drying for 16 hours at 100° were twice re-extracted with water and again dried. The results are summarized in Table II.

Exposure to heat seems to be the vital factor in this phenomenon. Whereas there was little difference in the material when air-dried, alcohol-dried or dried at 40°, higher temperatures enhanced the amount of water-soluble material obtainable. Air-dried or alcohol-dried samples subsequently exposed to heat were similar in properties to those directly given the same heat treatment when wet. Wet samples which were alternately rapidly frozen and thawed showed no change in properties.

Results of a similar character were obtained when extracting agents other than water, such as dilute acids and alkalis were employed. The effect of exposure to heat results in some modification of the properties of a portion of both components of isolated plant celluloses, which is manifest in increased susceptibility to extracting and hydrolysing agents. Moreover, this effect may be demonstrated repeatedly upon the same sample. The increments of water-soluble material become smaller, but are still appreciable after many treatments. In Fig. 2 the amounts removed from two samples of cellulose alternately dried and extracted are shown. Over the first twelve treatments the xylan

Table II. *Loss on water extraction of oat straw cellulose dried by various means.*

All results expressed on basis of 100 g. oven-dried material.

Series	Treatment of cellulose	Total loss		
		Extraction I	Residue from I, dried 16 hr. at 100°	Residue from II, dried 16 hr. at 100°
			Extraction II	Extraction III
A	Wet	4.3	6.2	8.7
B	Air-dried; room temperature (moisture content 7.53 %)	4.7	6.5	9.0
C	Alcohol-dried, room temperature	5.2	7.5	9.1
D	Dried at 40°	5.3	7.9	9.9
E	Dried at 60°	6.3	9.0	11.6
F	Alcohol-dried; put in benzene, heated under vacuum at 80° for 10 hr.	6.2	7.6	9.5
G	Dried at 80°	6.6	9.3	11.3
H	Oven-dried, 100° for 8 hr.	6.8	11.6	13.6
I	Air-dried, then heated at 100° for 16 hr.	7.6	9.7	14.0
J	Alcohol-dried, then heated at 100° for 16 hr.	7.1	10.9	14.2
K	Vacuum-dried, at 100°	8.1	10.2	12.8
L	Dried at 120° for 16 hr.	8.9	11.9	13.3
M	Dried at 150° for 16 hr.	9.6	11.7	14.2

Cellulose contained initially 32.3 % xylan.

removed accounted for about half the total loss and rather less after 20 treatments, at which point 80 % of the xylan had been removed, as compared with a loss of about 25 % of the "true cellulose". The xylan or cellulosan is therefore much the more susceptible fraction. Concurrently a distinct change in physical properties was observed on repeated extraction, the cellulose residue becoming less cohesive and losing the slightly gelatinous property which caused it originally to dry to a close hard mass, being instead light and friable.

Whilst the effect of heat on plant celluloses is to cause the formation of some water-soluble material, and whilst this effect can be repeated apparently indefinitely, it must be pointed out that pure cotton cellulose does exhibit the same phenomenon, but to a much smaller extent. An unbleached cotton yarn from

Table III. *Loss on water and citric acid extraction of cotton cellulose, oat and wheat straw cellulose after drying at 100° overnight.*

Expressed on 100 g. cellulose (oven-dried basis).

Extraction	Water: 15 min.			0.5% citric acid: 1 hr.		
	Cotton	Oat straw	Wheat straw	Cotton	Oat straw	Wheat straw
I	3.6	6.8	7.3	7.6	13.6	11.8
II	4.3	11.6	9.4	7.8	21.2	17.6
III	4.9	13.6	11.0	8.4	26.6	23.3
IV	5.2	17.1	13.0	8.7	28.8	25.1

high quality long fibre Egyptian cotton was treated with water and 0.5% citric acid for comparison with cereal straw celluloses. The results are given in Table III.

The isolated celluloses of cereal straws, containing as they do some 20% xylan, are clearly much more affected by heat-drying than is pure cotton cellulose entirely free from xylan. Since it is evident that the water-soluble material formed cannot be solely accounted for from the xylan, the inference is that the presence of cellulosan weakens the whole cellulosic aggregate and causes a greater degree of susceptibility to the effect of heat.

The nature of the water-soluble material.

The aqueous extract obtained on boiling an oven-dried cellulose preparation is only slightly reducing. For example, 1 g. oat straw cellulose extracted after oven-drying lost 68 mg. The extract titrated by the Shaffer-Somogyi [1933] micro-method contained reducing groups equivalent to 0.4 mg. glucose, a reducing value of 0.58% on the material extracted. Having established that the water-soluble material produced by drying is not hydrolysed by aqueous extraction, preparations were made from various isolated celluloses. The dried cellulose was boiled with water for 15 min. and the extract, after filtration, concentrated to a small volume under reduced pressure. To this straw-coloured liquid 5 vol. of alcohol were added, the white precipitate which formed being washed with acid alcohol, dried with increasing concentrations of alcohol and finally in a desiccator over fused zinc chloride. The preparations were slightly greyish white in colour, very light and hygroscopic.

Analyses showed that the preparations contained both hexose and pentose units as would be expected and some uronic acid groups. The anhydropentose content varied from 60 to 75% and uronic acid anhydride from 5 to 8% according to the source. Acidic groups were titratable with $N/50$ NaOH using thymol blue as indicator. A study was made of the rate of hydrolysis of certain of these preparations, the reducing sugar formed being titrated by the Shaffer-Somogyi micro-method, with the modification that the time of heating was increased to 25 min. since the shorter period recommended for glucose did not give complete recovery of xylose. The initial reducing values before hydrolysis were low, but rather variable, from 0.17 to 3% calculated as glucose. Hydrolysis by boiling with dilute mineral acids was rapid, but the recoveries of sugar were not very satisfactory. The shape of the hydrolysis curves did not suggest that there was a resistant portion unattacked by any of the acid strengths employed, but rather that the incompleteness of the recovery must be ascribed to destruction of sugar. It is known that high yields of xylose from xylan are difficult to obtain [Heuser & Brunner, 1922; Heuser & Jayme, 1923] and that the decomposition of pentoses during the hydrolysis of pentosans is greater than in controls of pure sugar similarly heated with acids. Since all the preparations were relatively rich in xylan, destruction of xylose probably accounted for the low sugar yields. The recovery of sugar from two preparations from oat straw cellulose is shown in Table IV.

Since a strong probability existed that these preparations were not homogeneous, an attempt was made at separation by precipitation at different alcohol concentrations. Four fractions, A, B, C, D, were obtained at 48, 65, 85 and 95% alcohol concentrations respectively. The yields from 90 g. oat straw cellulose were A, 1.6 g.; B, 0.39 g.; C, 0.14 g. and D, 0.04 g. The furfuraldehyde yields indicated the xylan contents to be respectively 70.5, 43.4 and 52.8%, the amount of D available being too small for any determination. The initial

Table IV. *Hydrolysis of water-soluble material from oven-dried oat straw cellulose.*

% sugar liberated expressed as apparent glucose.

Acid concentration	Time	Prep. 1	Prep. 2
		Xylan content 68.5 %	Xylan content 73.4 %
5% H_2SO_4	5 hr.	88.3	89.2
2.5% H_2SO_4	3 hr.	82.9	89.8
1% H_2SO_4	3 hr.	82.8	86.5
10% oxalic	3 hr.	93.1	85.9
2.5% H_2SO_4	1 hr. at 1 atmos.	84.2	85.6
72% H_2SO_4	2 hr. cold, diluted to 3% and boiled 2 hr.	79.7	81.5
3% HNO_3	1 hr.	70.7	—

reducing value of the main fraction A was 1.09 % in terms of glucose. The hydrolysis curves obtained with 10 % oxalic acid and 0.15 N H_2SO_4 were similar in shape, and the recoveries of sugar again unsatisfactory.

Evidence that the main fraction A obtained at the lowest alcohol concentration was still not homogeneous was given by separation by other means. A small amount was dissolved in cold 1 % NaOH and Fehling's solution added. The blue precipitate which formed, after washing with 50 % alcohol, was suspended in alcohol containing HCl and washed free from copper. This precipitate was found to yield furfuraldehyde equivalent to a xylan content of 86 %. The yield however was small and did not represent all the xylan in the sample taken. A similar partial separation of xylan was effected by adding alcohol to the alkaline filtrate from the copper precipitation above. A fraction was obtained at an alcohol concentration of 50 % which on subsequent washing and drying was found to contain 84 % xylan. Both procedures gave a fraction richer in xylan than the original preparation, which might be held to substantiate the view that the hexosan and pentosan are not combined.

The alcoholic filtrates from the precipitation of the water-soluble substance contained a small quantity of material not precipitated even at an alcohol concentration of more than 95 %. This was obtained by distillation under reduced pressure to a very small volume which was then made up to 10 ml. The solution was golden brown in colour and strongly reducing. On hydrolysis with acid the reducing value increased only about four or five times, an indication that the molecular size of this fraction must have been small. Figures for three such residues are given in Table V.

Table V. *Reducing power of alcohol-soluble fraction.*

	Expressed as mg. glucose.		
	I	II	III
Direct	48.3	37.5	33.0
Hydrolysed for 1 hr. with 5% H_2SO_4	180.0	111.0	—
Hydrolysed for 3 hr. with 2.5% H_2SO_4	—	—	142.0

Changes in the cellulose as a result of drying.

There are few tests that can be applied to cellulose itself to obtain information bearing on the effect of heat-drying. Degradative changes are usually accompanied by an increase in the copper number as a result of the production of

reducing groups. Such an increase was observed, the method employed being the Heyes [1928] modification of the Schwalbe-Braidy procedure. A small concurrent increase was found in the yield of CO_2 on distillation with 12 % HCl presumably owing to the production of uronic acid groups. It is not certain, however, that all the CO_2 given by cellulose is due to this grouping. The figures are given in Table VI.

Table VI. *Changes in copper number and CO_2 yield on drying wheat cellulose.*

Treatment of cellulose	Copper number*	CO_2 number†
Wet	0.60	0.30
Air-dried	0.75	0.43
Oven-dried, 16 hr.	0.83	0.45
Oven-dried and extracted with water twice, then air-dried	0.98	0.46
Oven-dried, 48 hr.	2.07	—

* Weight of copper reduced by 100 g. cellulose (dry).

† Yield of CO_2 on distillation of 100 g. cellulose (dry).

The changes, though small, are in the same direction as observed in the production of oxycelluloses and are, no doubt, of a similar type.

Discussion of the above observations.

This effect of heat drying of plant celluloses does not appear to have been directly recorded before, though in a brief paper by Sherrard & Blanco [1932] some experiments are given in which the same phenomenon undoubtedly occurred. They showed that from white spruce cellulose, kept wet, some material could be removed by prolonged boiling with water, but after drying the losses from the cellulose were heavier. Partial hydrolysis due to the long period of boiling was however the chief factor concerned in their experiments. From the extract, by concentration and precipitation with alcohol, a white powder was obtained, rather similar to those prepared in this work, in that it was very hygroscopic and easily hydrolysable to sugars. Experiments on the drying of wood made by Campbell & Booth [1930; 1931] showed a slight increase in the hot water-soluble fraction as a result of treatment at 100° or 105° , a fall in the Cross & Bevan cellulose content and a definite increase in alkali-solubility. These changes may be in part accounted for by the effect of heat on the cellulosic fraction.

The observations described in this paper do not admit of any ready explanation on the basis of present theories of the arrangement of cellulose molecules. The removal of water by drying is likely to modify conditions within the micellae. Shrinkage will occur and strains be set up as a result of the withdrawal of the "packing" provided by the water molecules. This disturbance might be expected to make for an increased susceptibility to the hydrolytic action of boiling water or stronger reagents. The observed effect of heat is, however, more difficult to understand. Alcohol-dried and air-dried samples subsequently exposed to a temperature of 100° were considerably affected by this further treatment from which water relationships are excluded. The effect of moderate temperatures on cellulose has been little studied. Profound changes take place on prolonged heating; indeed, it has been stated that complete carbonisation may occur in the course of a few months at 120° . Knecht [1920] has shown a rapid loss of colour and strength in cotton exposed at 93° . Whatever be the nature of the changes caused by the application of heat, plant celluloses containing celluloseans are very much more affected than is pure cotton cellulose. Both the initial loss on extraction and the further increments obtained by repeating the treatment are

considerably larger than in the case of cotton. The extra amounts are only in part accounted for by the cellulosan removed. The "true" cellulose aggregate is therefore less resistant than cotton or is weakened in this respect by the presence of cellulosan molecules within the micellae. The preparations of water-soluble material obtained do not throw much light on the nature of the changes responsible for the production of this fraction. The presence of two components which are undoubtedly affected to a different extent by the treatments means that any preparation will be a mixture. The cellulosan fraction is more affected by heat treatment than is the "true" cellulose since the xylan content of the water-soluble preparations was about 70%. It must be presumed that the effect of heat is to cause a breakage of some of the cellulose chains, and probably but not essentially a breakage of the xylan too. Evidence for chain breakage is provided by the increase in "copper number" values.

At the same time, some oxidation undoubtedly occurs with the production of uronic groupings and possibly also of terminal carboxyl groups of the gluconic type which would not be included in the estimation by CO_2 yield. Without assuming definite breakage of the cellulosic chains as a result of heat, it is not possible to account for the production of further water-soluble fractions, apparently similar, on wetting and re-exposing to heat. The water-soluble material may be supposed to consist of cellulosic fragments still of considerable though variable size and partially oxidized, mixed with cellulosan or degraded cellulosan molecules. Fractionation of the water extract was undertaken since it seemed likely that cellulosic fragments of diminishing molecular size might be precipitated by increasing alcohol concentrations and that differences in these might be evident in the rates of hydrolysis. In fact, however, the major part of the material removed precipitated at a relatively low alcohol concentration. No information exists as to the limit in number of glucose units in a chain imposed by water-solubility. A very small portion of the extract was strongly reducing and, since hydrolysis increased the reducing value only three or four times, the chain length of this fraction was probably quite short. The reducing value of the preparations was low, but cannot be taken as a reliable or even approximate index of average chain length because, owing to oxidation, the terminal groups might well be carboxylic rather than aldehydic in nature.

These observations may be of practical importance in two directions. In the first place, there is the possibility that the drying of any plant material before isolation or determination of the cellulose in it may have so changed the balance between cellulose and cellulosan that the product obtained is not truly representative. It is not certain that *in situ* and in association with other cell wall constituents such a change would occur. If it does, this is clearly a normal process in senescence and would take place during the ripening of straw, for example. Experiments to test this point are at present inconclusive, owing to the difficulty of grinding fresh tissues to as fine a state of division as dried materials. In routine work, at present, cellulose determinations are carried out on air-dried rather than oven-dried material.

A further implication of this work lies in the decomposition of cellulose by micro-organisms. The fermentation of celluloses which contain cellulosan has been little studied, the substrate in most work of this type being the pure celluloses of filter-paper or cotton. Heat treatment of a plant cellulose by producing a water-soluble fraction might expedite attack on the aggregate. If higher temperatures, possibly up to 140° , were employed, such a preliminary "cracking" might result in a more effective and rapid decomposition. This point is at present under investigation.

B. HYDROLYSIS AND EXTRACTION OF CELLULOSE PREPARATIONS.

Although most celluloses consist of two components, cellulosan and "true" cellulose, no quantitative separation has ever been achieved. The properties of the cellulosans are, with certain limitations, those usually described for the encrusting polyuronide hemicelluloses. That is to say, they are soluble in cold dilute alkalis and hydrolysed to their constituent sugars by boiling with dilute mineral acids. Limitations are conferred by reason of their association with the cellulose. The purpose of the work to be described was to investigate the removal of the cellulosan fraction of some celluloses by hydrolysis with acids and extraction with alkali.

There are few references in the literature to any previous work on this subject, indeed, only one paper, by Hawley & Fleck [1927], deals directly with the action of dilute acids on isolated cellulose. They showed that wood cellulose is hydrolysed much more readily than cotton cellulose and claimed that from softwood cellulose all the mannan and most of the pentosan might be removed with ease. The effect of dilute acids on cotton cellulose, as followed by the liberation of sugar, was determined by Wohl & Blumrich [1921] who found that the acid extract filtered free of cellulose had an increased reducing value on further boiling, indicating the presence of some compound intermediate between cellulose and glucose. A number of workers have studied the hydrolysis of wood with dilute mineral acids at ordinary temperatures and under pressure. Miller & Swanson [1925] showed that as a result of such treatment the cellulose is much reduced, a portion being lost at very low acid concentrations. This easily hydrolysable portion, amounting to about 13 % of the cellulose, was, no doubt, largely cellulosan in nature.

Only recently has it been realized that the cellulose of plant materials and woods is much less resistant to the action of acid and alkali than is cotton cellulose. Any treatment other than in neutral solution should be avoided in handling materials from which the cellulose is to be prepared or determined, in order that the integrity of the cellulose may be preserved. The nature of the attack brought about by acid and by alkali is shown in the following experiments. In view of the observations on the effect of heat-drying, all preparations were kept wet, and the treatments to be described were carried out on the wet material, due allowance being made for the moisture content.

1. Hydrolysis.

The effects of hydrolysis were studied by treating small samples for various times under standard conditions. The residues after thorough washing and drying were weighed and used for furfuraldehyde determinations. Reducing sugars were estimated directly in the extract using the Shaffer-Somogyi [1933] micro-method. The acid extract was also further hydrolysed by boiling again for 3 hours after adjusting the acid concentration to 4 % in each case. The reducing values of the extract both direct and after rehydrolysis are expressed as apparent glucose, it having been determined that the curve for xylose with this reagent is almost identical with that for glucose. With this information a full picture of the changes taking place on hydrolysis may be obtained. In Tables VII and VIII respectively are given the results obtained on the celluloses of oat straw and oak.

Considering first the series on oat straw cellulose given in Table VII, this cellulose was found to undergo appreciable loss with acid as dilute as 0.1 % (*N*/50) and in a period as brief as half an hour. Hydrolysis was progressive with time in this and all higher concentrations. Three important facts emerge and may

Table VII. *Hydrolysis of oat straw cellulose with acids at the boiling point. Furfuraldehyde yield = 17.82% equivalent to xylan 27.62%.*

All results expressed on the basis of 100 g. original cellulose.

Conc. of H ₂ SO ₄ %	Time in hr.	Residue	Xylan in residue	Xylan lost	Hexosan lost (diff.)	Sugars in extract	Sugars on re- hydrol.	Xylan loss Total loss %
0.1	$\frac{1}{2}$	93.1	23.4	4.2	2.7	2.7	8.8	61
	2	89.3	21.4	6.3	4.4	4.2	10.2	59
	5	88.2	20.8	6.8	4.9	6.0	12.1	58
	16	84.4	17.1	10.5	5.0	10.7	13.7	67
0.25	1	87.3	19.8	7.8	4.8	4.5	9.9	67
	2	85.7	18.1	9.6	4.8	6.5	12.2	67
	3	84.6	17.5	10.1	5.3	7.4	12.5	66
	5	82.7	16.2	11.4	5.8	10.5	13.1	66
	16	79.4	15.0	12.6	8.0	14.8	14.2	66
1.0	$\frac{1}{2}$	85.6	17.1	10.5	3.9	5.8	11.7	73
	1	83.9	16.9	10.7	5.3	8.6	13.9	67
	2	82.8	16.3	11.3	5.9	12.0	14.6	66
	3	81.7	15.2	12.4	5.9	13.5	14.8	68
	5	81.3	14.1	13.5	5.2	15.9	16.9	72
	16	77.2	8.9	18.7	4.1	22.4	21.4	82
3.0	$\frac{1}{2}$	84.7	16.8	10.8	4.5	10.9	14.4	71
	1	82.8	15.2	12.4	4.7	14.2	15.8	72
	2	80.7	12.8	14.8	4.5	17.1	17.7	77
	3	79.1	11.5	16.1	4.8	18.0	18.9	77
	5	76.5	9.9	17.8	5.7	19.4	20.3	76
	16	72.0	5.6	22.0	6.0	24.9	25.5	79
5.0	$\frac{1}{2}$	81.6	14.4	13.2	5.1	13.3	15.4	72
	1	80.6	13.8	13.9	5.6	16.5	16.9	72
	2	78.1	11.7	16.0	5.9	19.2	20.3	73
	3	76.2	9.9	17.7	6.0	21.2	—*	75
	5	74.7	8.1	19.5	5.8	24.0	—*	77
	16	67.3	3.5	24.2	8.6	27.3	—*	74

* Not determined.

be enumerated at once. Firstly, there is no apparent break in the continuity of the reaction with any of the concentrations of acid employed. If the effect produced on the cellulose in any selected time is plotted against the acid concentrations smooth curves are obtained, giving no indication of a fraction or fractions particularly susceptible to removal. Secondly, in no case did the xylan removed account for the whole of the loss sustained by the cellulose. There was evidently a concurrent extraction of hexosan, which presumably has to be considered as an attack on the true cellulose, though this point will be discussed more fully later. Thirdly, the reducing values of the filtered extracts obtained with all the lower acid concentrations were such as to indicate that the material removed had not undergone complete hydrolysis to reducing sugars. On increasing the acid concentration of the filtered extract to 4% in those cases in which it was originally less than this, on further boiling, the reducing values increased until the apparent sugar content was approximately equivalent to the total loss in the natural cellulose. The agreements were not very precise, no doubt owing to the instability of xylose in dilute mineral acid as referred to in the previous section.

Reviewing the series as a whole, the xylan may be said to be fairly readily removable by dilute acids, the maximum loss of 87% of the total xylan being achieved in 16 hours with 5% acid. With lower concentrations and in shorter periods the amount was almost proportionately less. The same, however, is not

Table VIII. *Hydrolysis of oak cellulose with acids. Furfuraldehyde yield = 15.93%, equivalent to xylan 24.69%. Lignin = 0.79%.*

All results expressed on the basis of 100 g. original cellulose.

Conc. of H_2SO_4 %	Time in hr.	Residue	Xylan in residue	Xylan lost	Hexosan lost (diff.)	Sugars in extract	Sugars on re- hydrol.	Xylan loss Total loss %
0.1	$\frac{1}{2}$	92.3	20.2	4.5	3.1	0.5	1.9	59
	1	90.1	19.8	4.9	4.9	0.9	5.1	50
	2	88.5	18.8	5.9	5.6	1.7	6.8	51
	3	88.0	18.0	6.7	5.3	2.3	7.4	55
	5	86.1	17.7	7.0	6.8	3.6	8.4	50
	16	83.9	16.0	8.6	7.5	8.5	11.2	53
0.25	$\frac{1}{2}$	90.3	20.1	4.6	5.1	1.0	5.1	48
	1	89.7	19.1	5.6	4.7	2.0	6.5	54
	2	87.8	17.9	6.7	5.4	3.5	8.3	55
	3	86.1	17.6	7.0	6.8	5.8	9.9	50
	5	85.4	17.3	7.4	7.2	8.0	10.5	51
	16	81.1	12.9	11.8	7.1	12.9	11.6	59
1.0	$\frac{1}{2}$	88.1	17.5	7.2	4.7	3.0	7.8	61
	1	87.2	17.3	7.4	5.4	5.9	8.9	58
	2	84.9	16.1	8.6	6.6	9.1	11.7	57
	3	83.7	15.4	9.3	7.0	11.1	13.1	57
	5	80.8	13.4	11.3	7.9	14.9	15.2	59
	16	74.2	8.8	15.9	9.9	19.3	—*	62
3.0	$\frac{1}{2}$	86.7	16.4	8.3	5.0	7.9	10.6	62
	1	84.4	15.0	9.6	6.0	12.1	12.4	62
	2	81.1	13.0	11.7	7.2	15.1	16.3	62
	3	77.8	10.9	13.8	8.4	16.4	16.4	62
	5	74.7	8.5	16.2	9.1	20.2	—*	64
	16	69.3	4.2	20.5	10.2	24.6	—	67
5.0	$\frac{1}{2}$	84.5	14.8	9.8	5.7	11.2	11.0	63
	1	79.9	12.5	12.2	7.8	15.5	16.2	61
	2	76.9	10.1	14.6	8.5	18.4	18.3	63
	3	75.4	8.9	15.8	8.8	22.1	—*	64
	5	71.3	6.4	18.3	10.4	23.5	—	64
	16	64.9	2.8	21.9	13.2	25.5	—	62
10	$\frac{1}{2}$	76.2	9.9	14.8	9.0	10.8	—	62
	1	73.6	8.6	16.1	10.3	13.1	—	61
	2	68.8	5.8	18.8	12.4	15.0	—	60
	3	66.8	4.4	20.3	16.5	16.5	—	61
	5	60.2	3.1	21.6	18.2	16.5	—	55
	16	57.5	1.3	23.4	19.1	16.5	—	55

* Not determined.

true of the hexosan removed. The results suggest the presence of a little over 5% of hexosan material removable from the cellulose by acid. On prolonged boiling this amount was somewhat increased. The hexosan does not show the same progressive removal as the xylan and the ratio of xylan loss to total loss increased with time except in the case of very dilute acids when it remained more or less constant. The material removed had not undergone complete hydrolysis to reducing sugars, though hydrolysis must have proceeded to a very considerable extent, for in only one experiment was the reducing value as much as doubled on rehydrolysis of the extract. The loss of xylan and hexosan must be ascribed largely to hydrolysis and not simply to extraction.

The results on oak cellulose given in Table VIII lead to similar conclusions. Some differences in degree are discernible and these are manifestations of the individuality of the cellulose. The xylan was rather less readily removed by very dilute acids, and whereas in oat cellulose about 5% hexosan was removed concurrently with the xylan, that fraction of the oak cellulose was more susceptible,

and seemed to be progressively attacked. With low concentrations of acid, the hexosan removed was almost equal to the xylan, the ratio of xylan lost to total loss being about 50 %. With higher concentrations this ratio increased but was not so high as that given by the oat cellulose. In another respect, too, differences were noted between the two samples of cellulose, the filtered acid extracts obtained being less completely hydrolysed in most cases.

Attempts were made to carry out a similar series of hydrolyses on a softwood cellulose, and although the removal of the xylan component could be followed accurately, it was not possible to distinguish in the dilute extracts between the mannose from the cellulosan and the other hexosan material presumably from the cellulose.

2. Extraction.

The effect of treatment of some plant celluloses with alkali was studied similarly by heating small samples for various times under standard conditions. Special precautions were needed in washing, to ensure the removal of all alkali.

Table IX. *Extraction of celluloses with boiling NaOH solutions.*

All results expressed on the basis of 100 g. original oven-dried cellulose.

Cellulose and xylan content	Conc. of NaOH %	Time	Residue	Xylan in residue	Xylan removed	Hexosan removed (diff.)	Xylan loss Total loss %
Oak (24.09)	0.1	10 min.	92.5	20.5	4.2	3.3	56
		1 hr.	89.4	18.8	5.9	4.7	56
		5 hr.	85.0	17.6	7.1	7.8	47
	1.0	10 min.	87.9	17.5	7.2	4.9	59
		1 hr.	82.2	16.6	8.1	9.7	45
		5 hr.	76.7	15.1	9.6	13.7	41
	4.0	10 min.	76.1	10.5	14.2	9.7	59
		1 hr.	72.3	8.6	16.1	11.6	58
		5 hr.	71.1	7.9	16.8	12.1	58
Wheat straw (21.52)	0.1	10 min.	94.7	18.5	3.0	2.3	57
		1 hr.	93.3	17.8	3.7	3.0	55
		5 hr.	87.4	15.3	6.3	6.3	50
	1.0	10 min.	90.7	16.3	5.2	4.1	56
		1 hr.	80.9	15.3	6.2	12.9	33
		5 hr.	66.0	8.2	13.3	20.7	39
	4.0	10 min.	80.1	10.4	11.1	8.8	56
		1 hr.	68.6	7.1	14.4	17.0	46
		5 hr.	62.9	7.0	14.5	22.6	39
Barley straw (24.04)	0.1	10 min.	84.1	14.9	9.2	6.7	58
		1 hr.	80.6	12.8	11.3	8.1	58
		5 hr.	76.5	10.4	13.6	9.9	58
	1.0	10 min.	78.9	11.6	12.4	8.7	59
		1 hr.	73.4	9.7	14.3	12.3	54
		5 hr.	68.1	9.1	15.0	16.9	47
	4.0	10 min.	72.2	6.3	17.8	10.0	64
		1 hr.	67.0	6.2	17.9	15.1	54
		5 hr.	62.8	5.6	18.4	18.8	50
Jute (14.73)	0.1	10 min.	95.1	12.2	2.5	2.4	51
		1 hr.	94.2	12.4	2.3	3.5	39
		5 hr.	90.1	11.3	3.4	6.5	34
	1.0	10 min.	93.1	12.3	2.4	4.4	36
		1 hr.	88.7	10.8	3.9	7.4	35
		5 hr.	85.3	9.9	4.8	9.9	33
	4.0	10 min.	85.7	8.6	6.1	8.2	43
		1 hr.	81.0	7.5	7.2	11.8	38
		5 hr.	80.2	7.2	7.5	12.3	38

Various concentrations of NaOH from $N/50$ to $2.5\ N$ were employed but results are presented only for 0.1, 1.0 and 4.0 % each at 10 min., 1 hour and 5 hours, since these conditions adequately cover the removal of the major part of the associated cellulosan. The celluloses studied included one from a hardwood, oak, two from cereal straws, barley and wheat, and one from a fibre plant, jute, the figures for all of which are given in Table IX. Alkali as dilute as 0.1 % brought about a considerable removal of xylan even in 10 min. from all celluloses, that of barley straw being particularly susceptible. The xylan was uniformly accompanied by some hexosan. With each concentration of alkali, long boiling removed hexosan at a greater rate than xylan, as a result of which the ratio of xylan loss to total loss falls with time. Not unconnected with this observation is the fact that in effecting the same total loss a higher concentration of alkali for a short period will remove more xylan than a lower concentration for a longer period. This is well shown in the oak series, in which the residue after extracting oak cellulose for 10 min. with 4.0 % acid was 76.1 % and that with 1.0 % acid for 5 hours 76.7 %. The xylan content of the former residue was, however, 10.5 % and of the latter 15.1 %. It follows that in the preparation of xylan from cellulose, a purer extract is likely to be obtained by the use of a strong alkali solution for a short period than by prolonged boiling with a more dilute solution.

The celluloses from the four different sources showed certain distinctive differences. The jute cellulose was more resistant to extraction than any of the other samples, and the losses from jute were due more to the removal of hexosan material than xylan. The celluloses from the cereal straws provided a distinct contrast, that from barley being much more susceptible to dilute alkali than that from wheat. In proportion, however, hexosan was more easily removed from wheat, and the ratios of xylan loss to total loss are consequently uniformly lower in the wheat series. Whether such differences are characteristic of these straws has not yet been determined. The results on oak cellulose provide the basis for a comparison of the effects of hydrolysis [Table VIII] and extraction. Prolonged boiling with acids produced a progressive attack on both components, that on the xylan increasing only slightly with time. As a result, on continued hydrolysis the ratio of xylan loss to total loss does not change appreciably. In alkaline extraction, on the contrary, the removal of hexosan increased faster than the removal of xylan with the result that this ratio falls.

Discussion of hydrolysis and extraction.

Treatment of a plant cellulose with hot dilute acids or alkalis results in the removal of cellulosan to an extent which depends on the concentration and the time. Concurrently, however, there is a removal of hexosan material often to an extent considerably greater than that of cellulosan. In no case, with hot reagents, is cellulosan removed unaccompanied by hexosan. Pure cotton cellulose with dilute acids and alkalis is known to undergo only very small losses under similar conditions. There is therefore in the celluloses from woods and plant materials a hexosan fraction far more susceptible than anything in cotton. The nature of this fraction and its relationship to true cellulose on the one hand and cellulosan on the other are not yet known. The existence of such a fraction has, of course, been recognized for many years, and in the evaluation of pulps a procedure is ordinarily adopted to exclude this less resistant material. By treatment in the cold with 17.8 % NaOH, a residue is obtained termed α -cellulose, which, whilst not usually free from xylan, is taken as representing the true resistant fraction available for manufacturing purposes. Bell [1932] has shown, in a study of the methylation of this fraction, that wood celluloses do not display uniformity

as individuals. The alkaline extract, if acidified, gives a precipitate known as β -cellulose. The larger part of the material extracted is not however precipitated on acidification, and it is termed γ -cellulose, the amount being obtained by difference. These fractions have never been critically investigated. It is unlikely that the proportions of α -, β -, γ -cellulose have any absolute significance since their isolation rests on a purely arbitrary procedure. The β - and γ -fractions presumably include the material removed by dilute acids and alkalis as described in this paper.

From these experiments certain limited deductions as to the nature of this less resistant hexosan can be made. It is apparently as easily removed by dilute alkalis as is the cellulosan and further is readily hydrolysed by dilute acids to give reducing sugars. True cellulose is little affected by dilute alkali or acids. The resistance of true cellulose to hydrolysis by acids is unusual, even amongst polysaccharides, and has never been satisfactorily explained. This property is presumably connected with the immense chain length of the cellulose molecule, but not with its micellar arrangement, for regenerated celluloses, of inferior organized structure, are not markedly more susceptible to acid hydrolysis. The inference is therefore that this less resistant hexosan fraction of such celluloses is of much less molecular size, and possibly of the same order or chain length as the associated xylan, the properties of which are virtually indistinguishable. This hexosan fraction might perhaps be more properly regarded as a cellulosan, instead of as a portion of the true cellulose, in which case the cellulosan of such materials as described above would have two components, a xylan and a glucosan, and in this be analogous to the cellulosan of the Gymnosperms, which consists of mannan and xylan. Such a distinction is however one of nomenclature only, and does not explain the nature of the material. The mechanism which produces the celluloses of plant materials and woods seems to be less perfect in its achievement than that of the seed hairs of the cotton plant. In the latter, the fibres are composed of molecules of great chain length and of the same general order of magnitude, so that the properties are uniform. In the former, molecules considerably shorter in length appear to be laid down with the longer true cellulose chains. These shorter molecules would comprise the less resistant hexosan material removed concurrently with the xylan fraction.

C. THE EFFECT OF DESTROYING MOLECULAR ORIENTATION.

The physical and mechanical properties of cellulose depend not only on the nature of the molecule, but on the state of aggregation of the molecular units. The fact that the molecules are oriented in one direction and stabilized laterally by secondary valency forces gives longitudinal strength. If cellulose be dissolved in one of the suitable solvents and subsequently precipitated, this regularity of arrangement is lost, and mechanical strength largely disappears. If cellulosans are present and are retained by secondary valency forces, the effects of disturbing the regularity of arrangement in such a cellulose should be an increased availability of the cellulosan fraction owing to the extensive reduction of forces operating laterally. This phenomenon has been observed in the case of the cellulosan of wheat straw cellulose. Two methods of solution were employed, in cuprammonium solution and by formation of the xanthate with CS_2 and alkali. A large preparation of the cellulose from wheat straw was made and, while wet, was divided into three portions. In view of observations on the effect of heat drying, it was decided to make comparisons of the regenerated cellulose both in a wet and oven-dried condition. One third of the cellulose was dissolved in cuprammonium solution by prolonged shaking, filtered free of lumps which were

broken up in a mortar until smooth, and the combined cellulose solution reprecipitated by pouring into a large volume of water containing more than sufficient HCl to neutralize all the ammonia present. The gelatinous precipitate obtained was washed by decantation and on a filter till free from copper. Half the product was then dried overnight at 100° and the remainder kept wet. The portion of the original cellulose for solution as a xanthate was treated with water and alkali to give a concentration of 17.5 % NaOH. CS₂ was stirred in, and the cellulose went slowly into solution to give a light yellow opaque liquid. It was diluted with 10 % alkali and kept overnight, giving then a clear red-brown viscous solution. The cellulose from this was regenerated by precipitation in a large bath of dilute H₂SO₄ and Na₂SO₄, a slow stream of the xanthate being run in from a tube of about 0.4 cm. diameter. In this way the cellulose was obtained in spongy irregular strings. After very thorough washing the product was divided into two portions, one being oven-dried and the other kept wet. The third portion of the original cellulose for use untreated as a control was similarly divided. In this way, six samples were obtained, untreated cellulose, wet and dried, cuprammonium cellulose, wet and dried and xanthate cellulose, wet and dried.¹ For comparison with these, similar series were prepared from two other celluloses not containing any cellulosan. One was a high-grade unbleached cotton yarn and the other filter-paper (Whatman No. 1). The origin of the cellulose of the latter was presumably wood, all the cellulosan having been removed by drastic acid and alkali treatment.

These samples were each given a number of treatments selected as a result of previous work for their suitability in providing information on the susceptibility of the cellulosan fraction. The treatments were:

- (a) 0.25 % NaOH
- (b) 1.0 % NaOH
- (c) 0.25 % H₂SO₄
- (d) Water: 2 boils for 20 min. each.
- (e) 3 % sulphite: 2 boils for 20 min. each.
- (f) 4.0 % NaOH: standing for 1 hour at room temperature.

The furfuraldehyde yield of the residue was determined on all wheat cellulose samples, both untreated and regenerated, and the reducing value of the extract from the two acid treatments.

The regenerated wheat celluloses from the cuprammonium and xanthate treatments were not identical in composition with the untreated wheat cellulose. In each case the basis of the solvent is an alkali, in the one ammonia (sp. gr. 0.920), and in the NaOH initially 17.5 % and later reduced to 13 %, as a result of which some of the xylan becomes extracted, and though reprecipitated on acidification is in a very fine condition and is carried away in the repeated washing that is necessary. This is particularly true of the cuprammonium product which reprecipitates in a highly dispersed condition. As a result, the xylan content of the xanthate product from wheat was 15 % and that of the cuprammonium rather less than 10 %, whilst the original cellulose contained 21.5 %. Such differences in xylan content might have invalidated the results had not the magnitude of the changes been great. The results of the extractions of wheat cellulose and its regenerated products are given in Table X, and those for cotton cellulose and filter-paper in Table XI. The results are expressed on 100 g. of the

¹ I am much indebted to my colleague Dr S. H. Jenkins for his assistance in the preparation and reprecipitation of the cuprammonium and xanthate celluloses used in this work.

Table X. *Extraction of wheat cellulose and regenerated celluloses.*All results except $\frac{\text{Xylan loss}}{\text{total loss}}$ expressed on 100 g. of the cellulose stated (oven-dried basis).

Treatment	$\frac{\text{Xylan loss}}{\text{total loss}}$				$\frac{\text{Xylan loss}}{\text{total loss}}$				$\frac{\text{Xylan loss}}{\text{total loss}}$				$\frac{\text{Xylan loss}}{\text{total loss}}$			
	Xylan in residue		Hexosan lost		Xylan in residue		Hexosan lost		Xylan in residue		Hexosan lost		Xylan in residue		Hexosan lost	
	Wet: untreated.	Xylan = 21.52%	Wet: untreated.	Xylan = 21.52%	Wet: untreated.	Xylan = 21.52%	Wet: untreated.	Xylan = 21.52%	Wet: untreated.	Xylan = 21.52%	Wet: untreated.	Xylan = 21.52%	Wet: untreated.	Xylan = 21.52%	Wet: untreated.	Xylan = 21.52%
Water	95.4	20.4	1.1	3.5	23	87.5	2.0	7.5	5.0	60	87.3	4.1	12.0	2.1	85	87
0.25% NaOH	88.3	18.3	3.2	8.5	28	85.2	2.1	7.4	7.4	50	82.8	3.1	11.9	5.3	69	66
1% NaOH	82.5	16.2	5.3	12.2	30	83.4	1.1	8.4	8.1	51	78.2	1.6	13.5	9.3	59	62
0.25% H ₂ SO ₄	90.6	16.7	4.8	4.6	51	87.6	1.4	8.1	4.3	65	84.2	4.1	11.0	4.8	69	77
2.5% H ₂ SO ₄	88.1	13.8	7.7	4.2	64	84.2	1.2	8.3	7.4	53	79.4	1.7	13.3	7.3	65	76
3% sulphite	95.0	20.5	1.0	4.0	21	88.0	2.8	6.7	5.3	56	85.3	4.4	10.6	4.1	72	78
4% cold NaOH	81.2	8.3	13.2	5.6	70	81.4	0.4	9.1	9.5	49	81.6	1.1	13.9	4.4	76	76
Dry: untreated																
Water	96.7	19.6	1.9	1.4	59	86.4	3.4	6.1	7.5	45	87.3	4.0	11.0	1.7	87	87
0.25% NaOH	81.0	15.5	6.0	13.0	31	80.0	2.8	6.7	13.3	33	81.9	3.0	12.0	6.1	66	66
1% NaOH	78.0	14.4	7.1	14.8	32	76.0	1.7	7.8	16.1	32	78.2	1.5	13.5	8.3	62	77
0.25% H ₂ SO ₄	88.3	15.4	6.1	5.6	52	86.8	3.6	5.9	7.3	44	84.2	2.9	12.1	3.6	77	76
2.5% H ₂ SO ₄	84.4	11.2	10.3	5.3	66	77.9	2.5	7.1	15.0	32	78.7	1.3	13.7	7.6	64	78
3% sulphite	92.4	18.8	2.8	4.8	36	84.8	4.7	4.8	10.4	31	86.2	4.3	10.7	3.1	78	76
4% cold NaOH	77.5	5.2	16.3	6.2	73	79.2	1.1	8.4	12.4	40	81.5	1.0	14.0	4.5	76	76
Dry: xanthate																
Water	96.7	19.6	1.9	1.4	59	86.4	3.4	6.1	7.5	45	87.3	4.0	11.0	1.7	87	87
0.25% NaOH	81.0	15.5	6.0	13.0	31	80.0	2.8	6.7	13.3	33	81.9	3.0	12.0	6.1	66	66
1% NaOH	78.0	14.4	7.1	14.8	32	76.0	1.7	7.8	16.1	32	78.2	1.5	13.5	8.3	62	77
0.25% H ₂ SO ₄	88.3	15.4	6.1	5.6	52	86.8	3.6	5.9	7.3	44	84.2	2.9	12.1	3.6	77	76
2.5% H ₂ SO ₄	84.4	11.2	10.3	5.3	66	77.9	2.5	7.1	15.0	32	78.7	1.3	13.7	7.6	64	78
3% sulphite	92.4	18.8	2.8	4.8	36	84.8	4.7	4.8	10.4	31	86.2	4.3	10.7	3.1	78	76
4% cold NaOH	77.5	5.2	16.3	6.2	73	79.2	1.1	8.4	12.4	40	81.5	1.0	14.0	4.5	76	76

cellulose concerned, and because of the losses of xylan in the course of preparation of the cuprammonium and xanthate products are not directly comparable with one another. Instead, the relative effects of the different treatments and the nature of the materials removed by those treatments must be compared.

Table XI. *Extraction of cotton cellulose and filter-paper and their regenerated products.*

Results expressed on 100 g. cellulose (oven-dried basis).

Treatment	Cotton cellulose						Filter-paper		
	Untreated		Cuprammonium		Xanthate		Filter-paper		
	Air-dried	Oven-dried	Wet*	Oven-dried	Wet	Oven-dried	Untr. air-dried	Cupr. wet	Xanth. wet
0.25% NaOH	94.2	92.0	89.0	92.9	97.3	95.6	97.1	96.8	95.1
1% NaOH	94.1	91.1	88.7	91.8	96.6	92.9	95.4	94.9	95.1
0.25% H ₂ SO ₄	94.4	93.2	87.3	95.5	98.4	93.3	97.8	98.2	95.2
2.5% H ₂ SO ₄	93.8	91.8	83.1	91.0	95.5	92.9	96.3	92.4	93.5
Water	98.3	95.1	93.9	97.3	98.6	95.9	98.8	99.1	95.8
3% sulphite	97.0	93.0	89.9	95.2	98.0	93.3	97.4	97.3	93.8
4% cold NaOH	96.5	95.3	96.6	96.3	98.7	96.8	97.6	97.3	95.2

* This sample was very gelatinous and became highly dispersed on subsequent treatment, so that some loss occurred on filtration.

The results on the wet xanthate-regenerated cellulose are more striking than those on the cuprammonium cellulose because less xylan was lost in the method of preparation. The differences between the various extracting and hydrolysing agents were smoothed out as a result of regeneration in both cases, so that hot water removed almost as much xylan as any other reagent, in marked contrast to

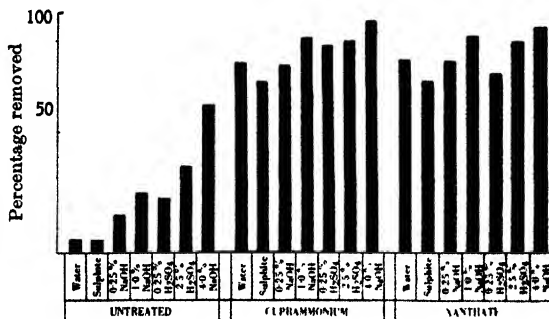


Fig. 3. Removal of xylan from untreated and regenerated cellulose.

its action on the untreated cellulose (Fig. 3). No equivalent increase in availability of hexosan material occurred. This is in accord with the results obtained on the celluloses not containing cellulosan given in Table XI. Neither filter-paper nor cotton cellulose on solution and reprecipitation showed any significant change in susceptibility to these various treatments.

The dried samples were very similar in properties to the wet, save that superimposed upon the changes brought about by solution and reprecipitation was also the effect of exposure to heat previously described. This appeared to be smaller in the regenerated celluloses than in the untreated material.

DISCUSSION.

It is believed that the cellulosan fraction of natural celluloses is retained by secondary valency forces. The association is, however, stronger than this type of linkage would normally provide. Additional stability is provided by the orientation of the cellulose molecular chains and their arrangement in micellae. If it is presumed that the cellulosan molecules are similarly oriented and distributed throughout the micellae, not necessarily only on the surface, then such an aggregate might be expected to have the properties of the natural celluloses as shown in this paper. A portion of the cellulosan may very readily be extracted with alkali in the cold, yet even prolonged heating with alkali fails to effect a complete extraction. This has long been a matter of comment. Heuser & Haug [1918], investigating a cereal straw cellulose, showed that many extractions with hot 6% NaOH resulted in a loss of only 80% of the xylan, and that even after drastic treatment under pressure at 140°–150°, 5–6% of the xylan remained. Similar results were obtained with the wheat cellulose used in this work. The xylan content, initially 21.5%, was reduced only to 5% by prolonged boiling with 10% NaOH, a loss of less than 80%. The hydrolytic effect of acid treatment is capable of removing this resistant fraction easily, and 3% H₂SO₄ at 100° for one hour eliminated this 5% residue. The distribution of xylan molecules through the micellae would account for such differences in extraction.

On the solution of the cellulose and subsequent reprecipitation the organized arrangement is largely destroyed, and the secondary valency forces between the cellulose and xylan molecules are no longer operative. As a result, the xylan becomes easily removable by reagents which previously had little effect upon it. In the untreated and oriented condition about 5% of the xylan only was water-soluble. After modification so that orientation was impaired nearly 80% of the xylan was removed under the same conditions. Cold 4% alkali then extracted well over 90%, a result that could not be achieved on the untreated material, even by prolonged boiling with alkali of higher concentration. No significant increase in the susceptibility of any hexosan material occurred, this change in properties being confined to the xylan.

SUMMARY.

1. The cellulose of most plants and woods differs from that of cotton in containing associated polysaccharides, known as cellulosans, which are tenaciously retained and must be considered as an integral part of the cellulose aggregate.

2. Heat-drying produces some change in the properties of both components of such a cellulose, which is manifest in an increased availability to extracting and hydrolysing agents.

3. The effect of heat treatment may be observed repeatedly on the same sample and must involve breakage of the cellulose chains, though the xylan fraction is affected to a much greater extent.

4. Preparations of the water-soluble material produced as a result of heat-treatment are mixtures which can be partially separated to give a portion of higher xylan content. Some oxidation undoubtedly occurs, and uronic groupings are present.

5. The xylan may be removed from celluloses by treatment with either acid or alkali, but a concurrent loss of hexosan material takes place in all cases.

6. In acid hydrolysis there is no apparent break in the continuity of the reaction. The reducing value of dilute acid extracts indicates that the material removed is not completely hydrolysed to reducing sugars.

7. Continued boiling with alkali removes hexosan at a greater rate than xylan, and in effecting the same total loss a higher concentration of alkali in a short period extracts more xylan than a lower concentration for a longer period.

8. Plant celluloses show considerable differences of behaviour towards hydrolytic and extracting agents and reveal distinct individualities.

9. By solution and reprecipitation of a cereal cellulose, the organized molecular structure may be destroyed, with the result that the xylan, which was initially extracted only to a small extent by water and dilute alkali, becomes almost completely soluble. No equivalent change in the properties of the hexosan material occurs.

10. These observations are in accord with the view that the cellulosan fraction of the cellulosic aggregate of plant materials and woods is oriented and participates in the micellae, being retained by secondary valency forces identical with those which obtain between parallel cellulose chains in pure cotton cellulose.

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CXCVII. THE COMPOSITION OF SOME LESS COMMON VEGETABLE FIBRES

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THE composition of many of the better-known vegetable fibres, which find commercial use in one way or another for textiles or cordage according to their properties, was recently determined, and at the same time an examination was made of the type of cellulose present [Norman, 1936]. It was shown that these fibres fall into two well-defined groups, one having only small amounts of xylan in the fibre cellulose, and the other in which the xylan is considerably higher. The so-called fine fibres, such as flax and ramie, fall in the first group, while the coarser fibres, such as manilla and sisal, must be included in the second. Through the courtesy of Sir Harry Lindsay and the Imperial Institute, it has been possible to examine a considerable number of additional fibres, mostly of tropical origin and not commonly met with. Few, in fact, find other than local usage, the isolation of the fibre from the tissue containing it being frequently carried out by primitive means only. The properties of a few of these fibres were such as to suggest that, if they were more easily obtainable, commercial development might be possible.

METHODS

As before, all samples were finely ground in a high speed mill wherever possible to pass a 60-mesh sieve. Sample 20 (Palmyra fibre) proved very resistant, being similar in this to coir, which it resembles. In this case the analyses had to be carried out on material far from homogeneous, which caused the cellulose determinations to be unsatisfactory.

The methods of analysis and their interpretation were described in the previous paper [Norman, 1936].

RESULTS

The analyses of these fibres, together with their common names (if any), origins, and botanical types are given in Table I. Many of them are somewhat similar in composition, containing cellulose in the neighbourhood of 75% and 5-10% lignin. There are some which deserve special comment. Sample 9, Nigerian jute, is almost identical with the Indian jute sample previously analysed, the only difference being a lower content of xylan. *Hibiscus cannabinus* (Sample 1) which is known as Bimlipatam jute contains only half as much lignin as true jute, but otherwise is not very different.

The two samples of *Urena lobata* (Samples 7 and 8) grown respectively in Uganda and India differ considerably in composition, the former being much lower in cellulose and a little higher in xylan than the latter. If the xylan is calculated as a percentage of the cellulose containing it (Table II), this is the more apparent. *Sida rhombifolia* from Sierra Leone and India (Samples 15 and 16) is very similar, the percentage of lignin in the former being a little greater.

Palmyra fibre (Sample 20) stands out from all the other fibres examined in its high content of lignin, resembling coir in that and in other characteristics.

Table I. Composition of fibres
All results expressed as % of oven-dry material

No.	Fibre and source	Botanical type*	Cellulose	Lignin	Total furfur-aldehyde	Cell furfur-aldehyde	Xylan in cellulose	Furfur-aldehyde from poly-uronides
1	<i>Hibiscus cannabinus</i> (Bimlipatam jute), India	a	76.61	5.95	11.30	8.90	13.8	2.4
2	<i>H. diversifolius</i> (Kanyamberege), Uganda	a	72.63	9.06	10.70	7.36	11.4	3.6
3	<i>H. rostellatus</i> (Kansambwe), Uganda	a	77.86	6.64	11.58	8.89	13.8	2.7
4	<i>Triumfetta rhomboidea</i> (Bwensambwe), Uganda	a	60.39	11.18	10.21	6.48	10.0	3.7
5	<i>T. tomentosa</i> (Binsambwe), Uganda	a	71.48	9.05	9.77	6.88	10.7	2.9
6	<i>Abutilon indicum</i> (Kifala), Uganda	a	72.18	10.59	13.75	9.62	14.9	4.1
7	<i>Urena lobata</i> (Kansambwe-Kasaja), Uganda	a	63.46	7.81	13.10	9.24	14.3	3.9
8	<i>U. lobata</i> , India	a	76.92	6.87	12.86	8.66	13.4	4.2
9	<i>Corchorus capsularis</i> (Green Jute), Nigeria	a	72.61	10.15	10.08	6.56	10.2	3.5
10	<i>Sterculia Barteri</i> (Eso), Nigeria	a	74.50	8.64	10.03	7.29	11.3	2.7
11	<i>Monodora brevipes</i> (Lakosin), Nigeria	a	72.58	10.75	10.59	7.04	10.9	3.5
12	<i>Grewia carpinifolia</i> (Itakan-Okoro), Nigeria	a	66.05	16.98	10.56	6.38	9.9	4.2
13	<i>Cola afzelia</i> (Obi Edun), Nigeria	a	75.60	9.85	12.19	8.87	13.7	3.3
14	<i>Abroma augusta</i> (Kowie), Sierra Leone	a	75.90	7.05	12.07	9.45	14.6	2.6
15	<i>Sida rhombifolia</i> (Helui), Sierra Leone	a	74.20	12.7	12.56	9.69	15.0	2.9
16	<i>S. rhombifolia</i> , India	a	74.84	10.22	13.21	9.88	15.3	3.3
17	<i>Honckenya ficifolia</i> (Napunti), Sierra Leone	a	74.35	10.66	10.8	8.49	13.2	2.3
18	<i>Sansevieria guineensis</i> (Bowstring hemp), Sierra Leone	b	75.06	7.31	12.35	7.95	12.3	4.4
19	<i>Malochra capitata</i> , India	a	73.41	8.27	12.5	9.72	15.1	2.8
20	<i>Borassus flabellifer</i> (Palmyra fibre), India	c (leaf)	63.50†	25.01	13.80	10.16	15.7	3.6
21	<i>Crotalaria juncea</i> (Sunn hemp), India	a	79.27	5.24	3.18	1.96	3.1	1.2
22	<i>Adansonia digitata</i> (Baobab fibre), Rhodesia	a (bark)	67.26	5.64	12.77	7.95	12.3	4.8
23	<i>Furcraea gigantea</i> (Mauritius hemp), Mauritius	b	79.83	4.83	13.24	9.85	15.3	4.4
24	<i>Ananas sativa</i> (Pineapple fibre), Sudan	d (leaf)	79.62	5.50	10.45	7.22	11.2	3.2
25	<i>Bromelia Magdalenae</i> (Pita fibre), South America (Colombia)	d (leaf)	76.35	6.93	9.67	6.80	10.5	2.9
26	<i>Bactris setosa</i> (Tucum palm fibre), South America	c (leaf)	81.54	7.42	5.13	3.10	4.8	2.0
27	<i>Eriodendron anfractuosum</i> (Kapok fibre),† Java	e	65.67	14.58	13.59	9.78	15.1	3.8

* Botanical type: a, bast fibres from dicotyledon stem; b, sclerenchymatous fibre bundles with residues of vascular bundles; c, vascular bundle with sheath of sclerenchymatous fibre; d, bundles of sclerenchymatous fibres accompanying vascular bundles; e, hairs from inner wall of fruiting capsule.

† This figure is less reliable than other cellulose determinations.

‡ This sample was extracted with alcohol-benzene before analysis.

Table II. *Xylan as % of cellulose*

Sample	Xylan	Sample	Xylan	Sample	Xylan
1	18.0	10	15.2	19	20.5
2	15.7	11	15.0	20	24.7
3	17.7	12	15.0	21	3.9
4	16.6	13	18.2	22	18.3
5	14.9	14	19.3	23	19.1
6	20.6	15	20.2	24	14.1
7	22.6	16	20.4	25	13.8
8	17.4	17	17.7	26	5.9
9	14.0	18	16.4	27	23.1

Although grinding was difficult and the cellulose figure consequently not so accurate as in other samples, the cellulose appears to be appreciably higher than in coir, which averages about 54 %. Coir, on the other hand, has the greater lignin content. The total furfuraldehyde yield and the percentage of xylan in cellulose are about the same in these two fibres.

Sunn hemp (Sample 21) from India is found closely to resemble the European hems previously examined. The cellulose is similar in containing only a small quantity of xylan, and this fibre must be added to the small group of low xylan type. *Sansevieria guineensis*, the so-called bowstring hemp, and *Furcraea gigantea*, Mauritius hemp (Samples 15 and 23) are not true hems, though the latter contains as much cellulose as Sunn hemp. The cellulose of both is relatively high in xylan, and these fibres must accordingly be allocated to the high xylan group.

Baobab fibre (Sample 22) is derived from the bark of a tree, and as might be expected is lower in cellulose than the majority of others examined, but at the same time it is not high in lignin, nor is the xylan in the cellulose as high as in most wood celluloses.

The analyses of Sample 25, Pita fibre from Colombia, differ considerably from those obtained on "Pita grass" and reported earlier. There is however some confusion in the description of this fibre. The results suggest what was suspected at the time from the unusually high cellulose content, that the samples of this fibre previously examined had been subjected to some purification process resulting in partial delignification. The xylan calculated as % of the cellulose is very similar in all the samples.

Tucum fibre or Tucum palm fibre (Sample 26) is separated from the leaves of this palm, common in the Amazon valley and along the coasts of Brazil. The fibre is high in cellulose and contains about 7 % lignin. The most noteworthy feature, however, is that the furfuraldehyde yield is low and the xylan in the cellulose under 5 %. This is unique in being the only leaf fibre yet found to be of the low xylan type. The fibre is fine, very strong and durable, but at present finds little other than local use in Brazil where it is utilized for fishing nets, bags and ropes.

Kapok (Sample 27) is found in the seed bolls of a tree, *Eriodendron anfractuosum*, but is entirely different in composition from cotton. The cellulose content is only about 65 %, and the cellulose is exceptionally high in xylan. From the appearance of the fibre it was not to be expected to be more lignified than jute or *Phormium*. Treatment with alcohol-benzene was found essential before analysis, and the results are expressed on fibre so treated.

DISCUSSION

It has been pointed out that the common fibres are divisible into two broad groups, on the basis of the xylan content of the cellulose present, and that this division does in fact separate the high quality textile fibres from those of coarser type. The additional fibres now examined permit only the addition of two, Sunn hemp and Tucum palm fibre, to the low xylan group. The remainder all contain upwards of 13 % xylan calculated on the cellulose (Table II). The degree of lignification is no indication of the type of cellulose to be met with, although no highly lignified fibres have been found to be of the low xylan type. There does not at present seem to be any reason why it is possible to group plant celluloses in this manner, the low-xylan group with 0-6 % and the high-xylan group with 13-25 % culminating in the hardwoods and straws. No cellulose with xylan between 6 and 13 % has been isolated in this laboratory although a very wide range of plant material has been examined.

There is clear evidence that the proportion of xylan changes during development, but none which suggests that the function of the tissue in any way affects the type of cellulose that may be present. Division into two groups according to xylan content may be an over-simplification, since no account is taken of the relatively short chain hexosan component that occurs with xylan in many plant celluloses. This ill-defined group about which information is not easy to obtain does not necessarily have the same distribution as the xylan.

SUMMARY

The composition of a number of less common vegetable fibres, mostly of tropical origin, is given. With two exceptions, Sunn hemp and Tucum palm fibre, the cellulose of all samples was found to contain over 13 % xylan.

The author is indebted to Dr S. E. Chandler of the Imperial Institute for the details of the anatomical characters of the fibres.

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CCCIV. THE DISTRIBUTION OF MANNAN
IN SOME GYMNOSPERMS.

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MANNAN has long been known to be widely distributed in woods, particularly in the softwoods or gymnosperms. Bertrand [1899] drew attention to the fact that whereas the hardwoods or angiosperms contain much xylan and little mannan, the softwoods contain more mannan than xylan and suggested that the mannan in a softwood occupies the same place as xylan in a hardwood. Schorger [1917] showed mannan to be present in 20 different species of softwoods in amounts up to 7.6%. Later it was realized that some of the mannan of softwoods is associated with the cellulosic fraction, and mannose was obtained from wood cellulose and wood pulps by hydrolysis [Lenze *et al.* 1920]. Heuser & Dammel [1924] found that the mannan of spruce pulp passes into the β -cellulose fraction, and Haggglund & Klingstedt [1927] demonstrated the presence of mannan in specimens of wood cellulose by a consideration of the optical rotations of the cuprammonium solutions. Actual preparation of the mannan from sulphite pulp was achieved by Hess & Lüdtkke [1928], who showed that its physical properties were similar to those of the mannan extracted from the ivory nut (*Phytelephas macrocarpa*).

The mannan associated with the cellulose of softwoods may be regarded as a cellulosan according to the nomenclature proposed by Hawley & Norman [1932], just as the xylan of hardwoods is so described. The xylan content of cellulose preparations is frequently determined in systems of analysis, so that a distinction may be drawn between "pentosans in cellulose" and "pentosans not in cellulose", but much less attention has been given to the mannan of softwoods. The purpose of the work described below therefore was to determine the distribution of the mannan in a few species of softwood and to examine the conditions of removal of the mannan associated with the wood cellulose.

EXPERIMENTAL.

I. *The determination of mannan.*

All methods of determination of mannan are based on the precipitation of mannosephenylhydrazone in the cold, and some modification of the procedure described by Bertrand [1899] is usually employed, such as that of Schorger [1917]. Briefly this consists of hydrolysing the wood by boiling for $3\frac{1}{2}$ hours with 5% HCl, neutralizing the extract, concentrating and precipitating the mannose by the addition of phenylhydrazine and acetic acid. Alternative methods involving extraction of mannan with alkali prior to hydrolysis have been shown to be inaccurate owing to incomplete extraction. It is not on record, however, that the quantitative aspects of the procedure recommended by Schorger [1917] have been studied, and in view of the fact that it is frequently necessary to determine mannose in low concentration this point was examined.

It was first established that the mannose produced on hydrolysis is stable to the mineral acid employed. No apparent fall in reducing value occurred when pure mannose was boiled with 5% HCl for periods up to 5 hours, the Shaffer-Somogyi micro-method being used.

The yield of hydrazone from different concentrations of mannose precipitated with (a) phenylhydrazine and acetic acid, and (b) phenylhydrazine hydrochloride and sodium acetate has been investigated, and the effect of the presence of sodium chloride examined. Some of the data from these experiments are given in Table I.

Table I. *Precipitation of hydrazone from different concentrations of mannose under varying conditions.*

Phenylhydrazine : sugar = 1 : 10. Stand at room temperature.

Conc. of mannose %	Sodium chloride added %*	Hours	Mannose recovered %†
1.44	12	6	93.4
1.20	12	6	92.4
1.20	—	6	91.8
0.62	9	6	92.4
0.60	—	6	81.5
0.60	—	2	69.0
0.56	12	6	90.0
0.37	16	6	86.0
0.30	—	2	51.9
0.30	—	6	71.7
0.30	9	6	83.9
0.15	—	2	8.0
0.15	—	6	38.0
0.075	—	4	Nil
0.075	—	6	Nil

10% phenylhydrazine hydrochloride solution containing equivalent sodium acetate.
10 ml. sugar solution used in each case.

Conc. of mannose %	ml. phenylhydrazine-HCl solution	Hours	Mannose recovered %†
2.1	5	6	94.0
2.1	10	6	95.4
2.1‡	5	6	Nil
1.5	5	6	94.7
1.2	5	6	95.3
1.2	5	24	94.9
1.2	5	6§	91.7
1.2	5	3	93.2
0.6‡	5	6	Nil
0.5	5	6	88.3
0.38	5	6	86.5
0.28	5	4§	70.5
0.28	5	4	75.2
0.13	5	6	24.0
0.06	5	6	Nil

* The significance of the concentration of NaCl added will appear later. The amounts roughly correspond to volume of 5% HCl selected for hydrolysis of mannan and final volume after concentration.

† Factor for calculation: hydrazone \times 0.66 = mannose.

‡ With addition of 0.9 g. NaCl = 6% NaCl on final concentration.

§ Solution heated to 75° immediately after addition of phenylhydrazine hydrochloride, then cooled to room temperature.

Several important points emerge. Either with free phenylhydrazine or the hydrochloride the recovery of mannose is dependent on the initial concentration of sugar in the mixture and only approaches a quantitative figure when the mannose concentration is about 1% or more. At very low concentration no precipitate forms. The time of standing must be longer than the 2 hours recommended by Schorger [1917], but 6 hours appear to be sufficient if the mixture be frequently shaken. It has recently been claimed that, in low concentrations of mannose, it is necessary to allow 3 weeks for the precipitate to form [Nishida *et al.* 1936]. No advantage was gained by heating to 75° after addition of the phenylhydrazine, though the precipitated hydrazone appeared sooner. The effect of the presence of salt is difficult to understand. With phenylhydrazine in the proportions stated, the presence of salt increases the recovery of mannose so that the limit of mannose concentration for 90% recovery or better is lowered to 0.5–0.6%. Using the hydrochloride, and under conditions in which the excess of phenylhydrazine is distinctly less, the addition of salt prevents the formation of any precipitate even on standing for 24 hours.

In view of these findings, some of the figures given by Schorger [1917] for the total mannan content of wood cannot be considered reliable. If, as stated, only 10 g. of wood were used for hydrolysis, and the final precipitation was carried out in 150 ml., the recoveries of mannose must have been incomplete in many cases.

The mannan content of pulps has been determined by Klingstedt [1933], who avoided the difficulties brought about by low concentrations by hydrolysing the pulp completely with 72% sulphuric acid, boiling after dilution and concentrating to a syrup after removal of sulphuric acid. Even so the conditions of the final precipitation do not result in recoveries of mannose so nearly quantitative as obtained in this work.

There is, however, need for further investigation of the precipitation of mannose as the hydrazone in order to obtain a more sensitive method suitable for woods. It was hoped to employ the iodimetric titration devised by Ling and Nanji [1921] for the determination of small quantities of the hydrazone, but the incompleteness of the precipitation rendered this impossible. Substituted phenylhydrazine derivatives might prove more suitable in low concentration and will be tested.

II. *The distribution of mannan in wood.*

The method adopted for the determination is as follows. 10–15 g. of finely ground wood, or 35–40 g. of wet wood cellulose, are boiled with 100 or 150 ml., 5% HCl under reflux condenser for 3½ hours, the residue after filtration on cloth being thoroughly washed twice with about 300 ml. hot water. The combined extract and washings, after cooling, is neutralized with NaOH, made just acid with acetic acid and concentrated to about 25–30 ml. The concentrated solution is filtered into a small marked flask and the filter paper washed till the volume is made up to 50 ml. (or 75 ml. in some cases). After cooling 2.5–4 ml. phenylhydrazine and up to 5 ml. 50% acetic acid are added. The flask is closed and allowed to stand for 6 hours at room temperature with constant shaking. The final volume is noted, the hydrazone is filtered off on a Gooch crucible, washed with about 50 ml. cold water followed by acetone, dried and weighed. $\text{Hydrazone} \times 0.6 = \text{mannan}$ or $\times 0.66 = \text{mannose}$.

A bulky precipitate due to the salting out of phenylhydrazine acetate may form if too much acetic acid is used, and additional water may have to be added. A small amount of this precipitate has no influence on the hydrazone yield and is removed on washing with water.

In all determinations the results have been rejected if the final concentration of mannose in the solution from which the hydrazone was precipitated fell below 0.5%, and further determinations were made employing larger quantities of wood or cellulose. The period of 3½ hours' hydrolysis has been based on Schorger's observation and the knowledge that both encrusting hemicelluloses and cellulans are hydrolysed in that time. If small amounts of mannan remain unhydrolysed they cannot be detected by a second hydrolysis because they fall below the limits of quantitative precipitation. 5% H_2SO_4 has been employed for hydrolysis and the acid subsequently removed with barium carbonate. This process, though very desirable, results in losses of mannose.

To study the distribution of the mannan in wood, the total mannan and the mannan associated with the cellulose were separately determined (Table II), the cellulose preparations and determinations being made by the procedure described

Table II. *Distribution of mannan in wood.*

Wood	Expressed on wood %		Expressed on cellulose %		Expressed on wood %		
	Total mannan*	Cellulose	Mannan in cellulose*	Xylan in cellulose	Mannan in cellulose	Xylan in cellulose	Mannan not in cellulose
Sitka spruce (<i>Picea sitchensis</i>)	6.02 (0.89)	61.87	8.43 (1.53)	5.05	5.22	3.12	0.8
Canadian spruce (<i>Picea</i> sp.)	5.75 (0.98)	63.72	4.92 (0.86)	9.19	3.13	5.86	2.6
Douglas fir (<i>Pseudotsuga taxifolia</i>)	7.06 (1.17)	57.46	10.14 (1.72)	5.54	6.82	3.18	0.2
Silver fir (<i>Abies</i> sp.)	5.96 (0.63)	52.90	7.01 (0.93)	7.34	3.70	3.88	2.3
Pitch pine (<i>Pinus</i> sp.)	5.07 (0.92)	55.88	7.01 (1.21)	8.30	3.92	4.64	1.2
Louisiana Gulf cypress (<i>Taxodium distichum</i>)	2.97 (0.55)	50.55	3.84 (0.53)	7.99	1.94	4.04	1.0
African pencil cedar (<i>Juniperus procera</i>)	2.40 (0.52)	44.29	4.05 (0.65)	11.03	1.79	4.89	0.6
Redwood (<i>Sequoia</i> sp.)	2.62 (0.72)	49.08	4.77 (1.08)	11.61	2.34	5.70	0.3

* Figures in brackets give the mean concentration of mannose in the final solutions from which the hydrazone was precipitated.

by Norman & Jenkins [1933]. All results are the average of two closely agreeing determinations. In view of the fact that, as shown in Table I, completely quantitative recovery of mannose is not obtained under the conditions of precipitation employed, the figures must err slightly on the low side. The woods analysed may be taken as a representative group of softwoods. In all cases the major part of the mannan was found to be associated with the cellulose. The highest content occurred in Douglas fir, and in this wood, and in Redwood, virtually all the mannan was present as cellulosan. In the final column of Table II is given "mannan not in cellulose", obtained by difference. It is unlikely that this has any existence in fact as a true mannan, but is probably a constituent part of the encrusting hemicelluloses. As expected, no regularity was found in the proportions of mannan to xylan in the cellulose. The variation was very wide, from Douglas fir cellulose on the one hand, which contained nearly twice as much mannan as xylan, to African pencil cedar on the other, with more than twice as much xylan as mannan. The number of samples is insufficient to show whether

this may be a varietal difference, or influenced by age or tissue. Norman [1936, 1] has shown that the amount of xylan associated with the cellulose of rye grass increases with the age of the tissue. If there is any degree of constancy in the mannan/xylan ratio for particular species, this might prove a useful method of identification in certain cases. The cellulosan content of these softwood celluloses, as judged by the mannan and xylan contents combined (12–16 %), is notably lower than that of hardwood celluloses in which it is often over 20 %. This fact is of course represented in another way in the higher α -cellulose content of the softwood celluloses.

III. *Hydrolysis and extraction of mannan from softwood cellulose.*

The hydrolysis of wood with dilute mineral acids has been extensively studied, but few investigators have examined the behaviour of isolated celluloses. Hawley & Fleck [1927] reported that all the mannan and xylan could be easily removed from spruce cellulose by the action of dilute acid.

The purpose of the experiments described below was to study the relative behaviour of mannan and xylan to hydrolytic and extracting agents, since this has an important bearing on the form of association of the cellulosan with the true cellulose component of the wood cellulose. The wood employed was Silver fir, which seemed particularly suitable for this purpose inasmuch as the mannan and xylan contents of the cellulose are almost identical.

A large preparation of cellulose was made from this wood, and in view of the observation that drying may change the relationship between cellulose and cellulosan [Norman, 1936, 2], all treatments were carried out on the wet cellulose. The losses resulting from the different treatments were determined on small samples of cellulose under the same conditions as used for the larger amounts needed for mannan determination. The residues from these smaller quantitative experiments were later utilized for the determination of xylan by furfuraldehyde yield. In the case of the experiments on acid hydrolysis mannan was determined on the extract or the residue, whichever provided the highest concentration. It proved difficult to select such amounts as would keep the concentration above the limits necessary for maximum recovery. The results of hydrolysis with dilute acid are given in Table III.

Table III. *Hydrolysis of Silver fir cellulose with boiling dilute HCl.*

Mannan in cellulose 7.01 %. Xylan in cellulose 7.34 %.
Results expressed as % of oven-dry cellulose.

Treatment	Residue	Mannan in residue*	Xylan in residue	Hexosan removed
3% HCl 1 hour	84.91	4.16 (0.74)	4.78	9.68
3% HCl 3 hours	77.45	2.78 (0.44)	2.93	13.91
1% HCl 1 hour	90.09	5.29 (0.75)	5.16	6.01
1% HCl 3 hours	86.40	3.76 (0.42)	4.78	7.79
0.25% HCl 1 hour	94.94	6.62 (0.83)	5.84	3.17
0.25% HCl 3 hours	91.74	5.47 (0.67)	5.04	4.42

* Figures in brackets give the mean concentration of mannose in the final solutions from which the hydrazone was precipitated.

The removal of both mannan and xylan was far from complete even after boiling with 3 % acid for 3 hours. The mannan and xylan showed little difference in behaviour excepting perhaps to the lowest concentration, 0.25 %, towards which the xylan appeared to be more susceptible than the mannan. Attention is

drawn again to the last column of the table in which the amount of material removed which is not mannan or xylan, but presumably glucosan, is recorded. In every case this is greater than the combined loss of mannan and xylan.

Interesting differences in behaviour of the xylan and mannan were shown in cellulose exposed to cold and hot alkali (Table IV). The mannan appears to be

Table IV. *Treatment of Silver fir cellulose with NaOH both hot and cold.*

Mannan in cellulose, 7.01%. Xylan in cellulose, 7.34%.

Results expressed as % of oven-dry cellulose.

Treatment	Residue	Mannan in residue*	Xylan in residue	Hexosan removed
4% NaOH 2 hours (room temp.)	92.07	5.50 (0.90)	3.30	2.24
1% NaOH 2 hours (room temp.)	98.02	6.71 (0.94)	6.67	1.01
4% NaOH 20 min. (boiling)	85.43	2.30 (0.52)	4.96	7.48
1% NaOH 20 min. (boiling)	89.86	2.82 (0.56)	6.90	5.51

* Figures in brackets give the mean concentration of mannose in the final solutions from which the hydrazone was precipitated.

more resistant to extraction than xylan when cold alkali is employed, but distinctly less resistant to hot alkali. 1% NaOH at the boil for 20 min. removed 60% of the mannose and only 6% of the xylan whereas cold 4% NaOH extracted about 55% of the xylan as against 21% of the mannan. The significance of these differences is not apparent but is worthy of more extended study. In the preparation of mannan from cellulose the use of hot 1% NaOH for a short time would result in a purer extract than would be obtained with a higher concentration. The results are, of course, insufficient for generalization, but suggest that the stabilities of mannan and xylan are of the same order and the forms of association with the cellulose of a similar type.

SUMMARY.

1. The precipitation of mannose as the phenylhydrazone is not quantitative at very low concentrations. Almost complete recovery may be obtained if a certain minimum concentration is exceeded. Salt may aid precipitation if a large excess of phenylhydrazine is present.
2. Conditions suitable for the determination of mannan in woods and wood cellulose are described.
3. The major part of the mannan in softwoods is associated with the cellulose. Considerable variation is found in the proportion of mannan to xylan occurring in the cellulose.
4. Mannan may be removed from cellulose by dilute acid hydrolysis under conditions similar to those in which the xylan is extracted. At the same time there is a considerable loss of hexosan. The mannan and xylan are affected to different extents on treatment of the wood cellulose with alkali.

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A PRELIMINARY STUDY OF THE PHYSICAL SIGNIFICANCE OF CERTAIN PROPERTIES MEASURED BY THE CHOPIN EXTENSIMETER FOR TESTING FLOUR DOUGHS

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Introduction

In a number of recent publications a method has been described for measuring the viscosity (η) and rigidity modulus (n) of flour doughs (see Schofield and Scott Blair, 1932, 1933, 1933a, 1937). These properties are not independent of stress or strain, but it has been shown that if suitably standardized conditions are used, values of η and n (which can be expressed in absolute units) can be obtained which give direct information about the capacity of the dough to make good bread under English bakehouse conditions.

The most important properties of a dough for making English bread are—

Spring which is measured by the ratio of viscosity to a power of the modulus,

Extensibility which is normally associated with a relatively small fall in viscosity with rising stress, and

Tolerance which means that viscosity should not be too sensitive to changes in moisture content and fermentation-time.

This work was carried out jointly by the Physics Department, Rothamsted Experimental Station, Harpenden, England, and the Research Association of British Flour Millers, and is being published in a series of joint papers (Halton and Scott Blair, 1936, 1936a, 1937a).

Recent co-operation between these institutions and the Centre de Recherches Agronomiques, Versailles, France, has made possible an extension of the principles developed to a study of French conditions and a comparison with the Chopin extensimeter (Chopin, 1921, 1921a) now widely used in commercial flour and wheat testing throughout the continent of Europe.

In the Chopin technique, a bubble is blown in a thin strip of dough. The maximum pressure (P) and the square root of the volume attained before the bubble bursts (G) are automatically recorded. The air pressure required to cause the bubble to form is applied by means of a falling column of water, *i.e.*, at a regular but decreasing rate. The pressure in the bubble rises to a maximum, at which point the walls of the bubble are said to become permeable to air, although it continues to expand for some time longer before final rupture and collapse occur. Although the pressure passes through a maximum the surface of the bubble is still increasing, and there is no evidence that the stress per unit area does not continue to rise up to the final rupture. The viscosity of flour dough falls with rising stress (structural viscosity) but rises with increasing deformation (work-hardening (see Schofield and Scott Blair, 1932)). The variation of viscosity during the production of the bubble will thus be complex, but at the point of final rupture the viscosity may be defined as the momentary shearing stress divided by the rate of change of non-recoverable deformation. Since the rate at which air is supplied to the bubble is predetermined, it seems probable that the value of P should be correlated primarily with the viscosity of the dough.

Bakers use different quantities of water with different flours, so that the viscosities of doughs used in the bakehouse do not differ nearly as widely as do those of the Chopin test doughs which are all made up to contain the same quantity of water. Hence a flour of high water-absorbing capacity gives a dough of high viscosity when made up with the standard amount of water, whereas a flour of low absorption produces a dough of low viscosity. This suggests that under the standard conditions of the Chopin tests, P , being related to viscosity, should be a measure primarily of the water-absorbing capacity of the flour.

The significance of G is not so easy to predict, but the following line of argument is suggestive:

After the bursting of the bubble, the walls recover to about half their fully distended area. The total deformation is thus divisible into two parts, (a) recoverable deformation (σ_r), and (b) non-recoverable (σ_n). σ_r will be defined by the ratio of the shearing stress S to the shear modulus η . It therefore follows that the lower the modulus the bigger will be σ_r for a given stress. Now the higher the viscosity the bigger will be the stress under the arbitrarily fixed rate of application of deformation (remembering that viscosity is defined as $S/(\text{rate of change of } \sigma_n)$) which will, in its turn, make for a bigger extension. The higher the viscosity, the greater the proportion of elastic to total deformation. It thus appears that a high η and a low η , which have

already been shown to give a big elastic recovery, are also the predominant factors in producing a high value of G (see Halton and Scott Blair, 1936). This, however, presupposes that the dough is reasonably extensible. It is clear that a "short" dough will tear before either a high pressure or extension can be produced. Now it has been shown that shortness is correlated with the rate at which η falls with rising stress (Halton and Scott Blair, 1936a), and some measure of extensibility is obtained from the ratio η_h/η_l (where η_l is viscosity at low stress (normal stress), and η_h at high stress).

It is appreciated that this argument is not strictly quantitative, but bearing this in mind, we can proceed to write a generalised equation of the type:

$$\text{Equation (1)} \quad G = (f) \frac{\eta_l^a \cdot \eta_h^c}{\eta^b \cdot \eta^d} \quad \left\{ \begin{array}{l} \text{where } a, b, c \text{ and } d \text{ are} \\ \text{unknown powers.} \end{array} \right.$$

The baking value of flour has been shown to depend under English conditions, on suitable values of viscosity and shear modulus. The optimum relationship between these two properties, and the best conditions for their variation with other factors such as stress, age of dough, moisture content, *etc.*, have not yet been fully worked out; but although the requirements of the French market differ somewhat from the English, it is reasonable to suppose that whereas the relative importance of the different factors will not be the same, the same fundamental properties will be significant in both countries. In judging the potentialities of new varieties of wheat, it is not possible to do baking tests for lack of large enough samples, and the Chopin figures have to be taken as a criterion of value. It is thus of the greatest importance to find out what combination of physical properties the instrument really measures and a preliminary study of this problem forms the subject of the present note.

Experimental

Doughs made by the standard Chopin technique from some 35 flours of widely different origin were examined, measurements of P , G , η_h , η_l , and η being recorded. P and G were obtained from the Chopin diagram in the usual way. η_h was determined by measuring the rate of flow of a dough under a load¹ of about 80 to 85 Kg./cm.² through a narrow brass tube of approximately 5 mm. diameter and 5 cm. length. No attempt was made to convert η_h into absolute units, and it is realised that, as a measure of true viscosity, it is only approximate. η_l and η were determined by the technique already described by Halton and Scott Blair (1936, 1936a) at a shearing stress² of 1200 dynes/cm.²

¹ *i.e.*, a shearing stress of the order of 10^7 dynes/cm.².

² Since the technique is being described in an article in this journal (Cereal Chem., 14: 205), no further description of it will be given here.

In order to check that η_1/η_h was giving a measure of "shortness" as determined by feel, a spare portion of each dough was felt carefully before testing, and a note recorded.* It was afterwards found that the mean values of η_1/η_h corresponding to those notes were as follows:

TABLE I

Note	Number of doughs	Mean η_1/η_h
Very short	8	28
Short	10	22
Short-medium	9	16
Medium	5	13
Medium-extensible	1	12
Extensible	1	9
Total	34	

This shows a good general agreement between shortness as assessed by feel and η_1/η_h .³

In order to test the validity of an equation of the general type of (1), still further simplifying assumptions must be made. As a first approximation it is assumed that $a = d$ and $c = 1$. Hence, we can write:

$$\text{Equation (2)} \quad G = (f) \frac{\eta_h}{n^b}.$$

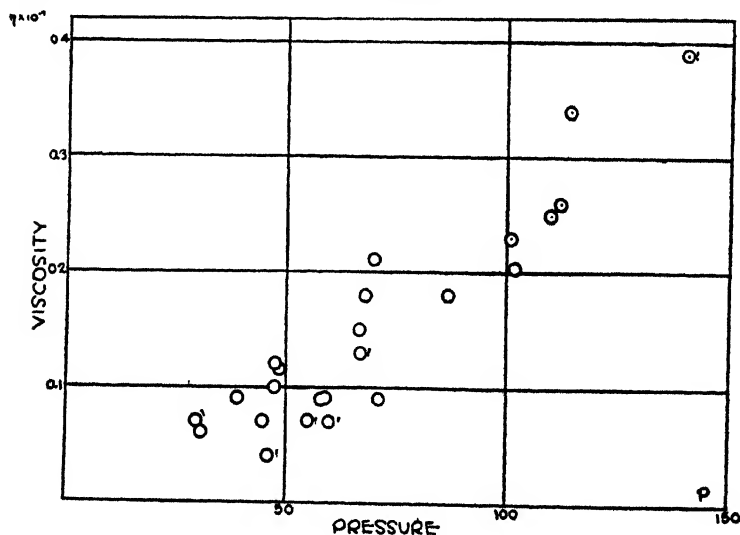


Fig. 1.

The data were examined to see whether any such correlation existed fitting different values for b , and it was found that the most satisfactory

* Since "shortness" does not have quite the same significance in different countries it should be stated that all assessments by feel were done by one of the authors (G.W.S.B.).

results were obtained when $b = 2.0$. This is interesting in view of the findings of Halton that η/η^* , which is a measure of spring, becomes independent of moisture content when x is given a value of slightly less than 2.0 for most flours. The results of the main experiment are shown in Figures 1 and 2. The general correlation between P and η

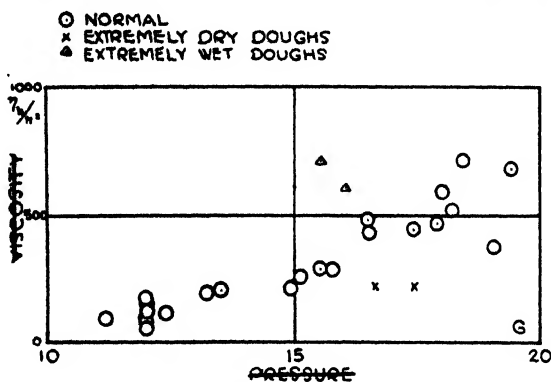


Fig. 2.

is clear from Figure 1. In Figure 2, values of G are plotted against η/η^* . The two samples marked (Δ) were exceptionally wet doughs, and the two marked (\times) were abnormally dry. It is clear that for doughs which are neither exceptionally wet nor dry, there is a good general correlation, and this is especially noteworthy when one considers how very many assumptions are made in deriving equation (2).

Conclusions

The Chopin extensimeter, used in the usual manner in which all doughs are compared at the same moisture content, measures three factors:

- (1) The maximum pressure attained when a bubble is blown in a strip of dough under standard conditions (P)
- (2) The square root of the maximum volume (G)
- (3) The area of the pressure-volume diagram (W). This is believed to depend partly on the property of the dough to allow gas to escape through it before final rupture and is therefore not considered in relation to viscosity and modulus measurements.

P is primarily related to the moisture absorbing capacity of the flour.

G is primarily related to a complex function of viscosity and modulus, but is approximately dependent on the product of a function known to be related to "spring" and one related to "shortness." Other factors undoubtedly enter to a lesser extent.

The baking quality of a flour depends primarily on spring and shortness, but it is not yet possible to say how far the composite function of them measured by the Chopin extensimeter corresponds with what is required for a good baking quality either under French or English conditions. This point requires further investigation.

Summary

A preliminary analysis of the physical properties of dough measured by the Chopin extensimeter indicates that water absorption capacity and a complex function of viscosity and modulus are the principal factors involved. Under the conditions of the test, the former is directly related to viscosity, and the latter depends on a complex mixture of "spring" and "shortness" which has been only partially resolved.

In view of the increasing use which is being made of the Chopin instrument as a criterion of wheat and flour quality independent of any baking test, the importance of a wider understanding of the nature of the factors measured is stressed.

In conclusion, one of the authors (G.W.S.B.) wishes to express his gratitude to M. Demolon, Inspecteur Général d'Agriculture de France, for the hospitality of the Centre National de Recherches Agronomiques de Versailles during the progress of this work, and to the Société pour la Vulgarisation des Engrais for the help which made possible his participation in it.

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The Relationship between Viscosity, Elasticity and Plastic Strength of a Soft Material as Illustrated by some Mechanical Properties of Flour Dough

IV—The Separate Contributions of Gluten and Starch

By R. K. SCHOFIELD AND G. W. SCOTT BLAIR

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In the earlier papers of this series (Schofield and Scott Blair 1932, 1933*a*, 1933*b*) the endeavour has been to give a quantitative description of the behaviour of flour dough under stress. Use was made of the equation

$$\frac{de}{dt} = \left(\frac{1}{n} \cdot \frac{dS}{dt} - \frac{d\alpha}{dt} \right) + \frac{1}{\eta} \cdot S,$$

which is the expression originally put forward by Maxwell with the addition of $-d\alpha/dt$ to take account of elastic after-effect. In this equation de/dt represents the rate of elongation of a cylinder of dough, and S the shearing stress which is one-third the longitudinal stress per unit area. The equation serves to define n , the modulus of rigidity, and η the viscosity, and enables these to be evaluated from experimental observations of e and S .

The behaviour of flour dough was shown to be consistent with the equation

$$t_r = \eta/n$$

for the relaxation time, and evidence has since been presented by Halton and Scott Blair (1936) to show that t_r is probably the most important single quantity determining the baking quality of a flour dough.

Valuable as this method of formulation has undoubtedly been, it does not throw much light on the physical mechanisms at work, its virtue being that it can be applied to any material that can be stretched. By its use a remarkable *formal* resemblance was demonstrated between the behaviour of flour dough and soft metals: both show work hardening, elastic hysteresis and elastic after-effect. It cannot be argued from this, however, that the physical mechanism is the same. To study this aspect of the problem other avenues of approach must be sought.

THE MECHANISM OF WORK HARDENING IN FLOUR DOUGH

The Gluten Network—An investigation of the behaviour of dough cylinders when extended to many times their original length has provided a clue to the explanation of work hardening.

The apparatus was a simplified form of that already described. The cylinder of dough originally 5 cm. long and about 0.7 cm. in diameter was floated straight out of the "moulding gun" on to the mercury bath which was provided with a lid lined with wet felt to prevent the dough from drying. Threads of sewing cotton were attached, one to each end of the cylinder, by means of small pieces of cork to which dough adheres readily. One thread was attached to a stress indicator, reading up to about 7000 dynes,* of stouter design than that used previously, and the other to a winch geared to a synchronous motor which enabled it to be moved steadily at a rate of 0.045 cm./sec.

In the first series of experiments represented by figs. 1, 5 and 6, the stress was recorded at intervals during the slow extension at the end of which the cotton was released from the winch. The dough cylinder was then allowed 5 min. for free contraction before the slow extension was repeated. In these figures the shearing stress per unit area (dynes/cm.²) is plotted against $\log_e l/l_0$, l being the length at the moment in question and l_0 the initial length (5 cm.).

The fact that the length of a dough cylinder which has completed its elastic recovery after being stretched always exceeds the original length, is most simply explained by supposing that the elastic elements in the dough are insecurely attached to each other. It might be expected that a general slippage would occur when a critical stress had been reached. This, however, is not the case in a normal dough. Fig. 1 shows clearly that although considerable flow took place in the first extension, very much less occurred during the subsequent extensions although somewhat higher stresses were recorded. The upward curvature at the higher strains which is such a pronounced feature of the curves is just discernible in fig. 7 of the paper by Schofield and Scott Blair (1933*b*) which was only carried to a strain $\log_e l/l_0 = 0.7$.

The general nature of the process appears to be something like that indicated in fig. 2, which represents the behaviour of six springs, three of which are securely linked at P , the other three at Q , while they are insecurely linked in pairs at R , S and T . The springs linked at S are only about half the length of those linked at R and T . Consequently, if each insecure link

* 15 cm. at 0.455 g./cm. = 6700 dynes.

will stand the same maximum stress, the link at *S* will snap before those at *R* and *T*. If only a small stress is applied (fig. 2*b*) all the links will hold, and the system when released will recover to the unstrained length (fig. 2*a*). But, once *S* has snapped (fig. 2*c*) the system will not recover to its length in

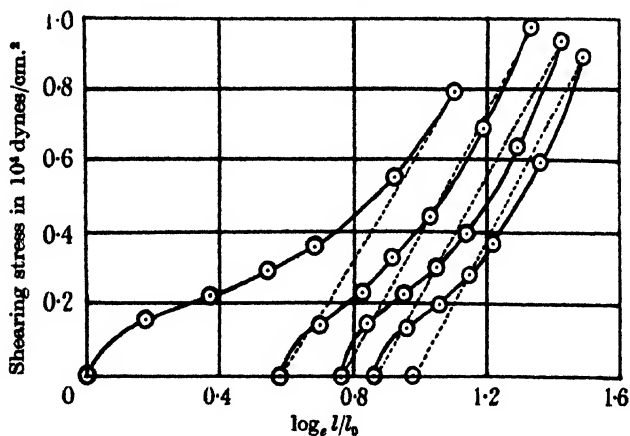


FIG. 1

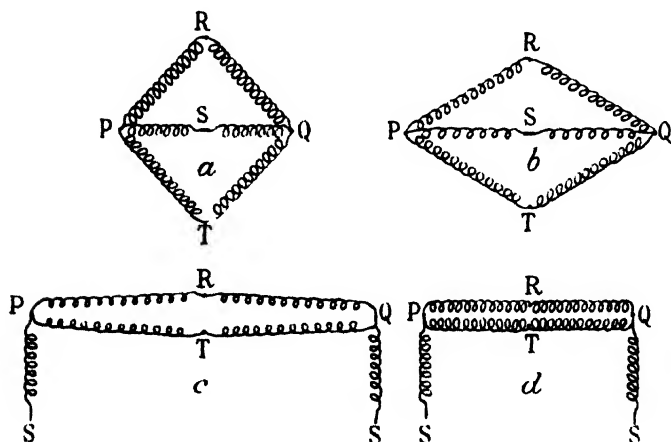


FIG. 2

fig. 2*a* but only to the condition shown in fig. 2*d*. The system has undergone a permanent elongation and has therefore "flowed". It must be remembered that the dough is to be pictured as formed of a very great many such systems. The more junctions that have broken, the higher must the applied stress be before there is any more breaking of junctions. Work hardening

may be envisaged either as a rise in yield value, or in viscosity, but in either case it is clear that the progressive snapping of individual links has produced hardening.

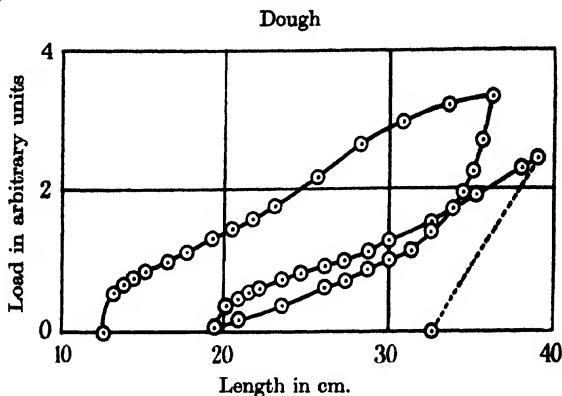


FIG. 3

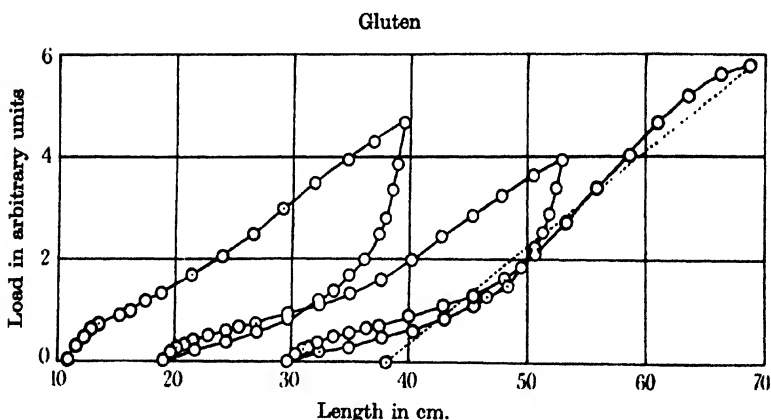


FIG. 4

For more easy comparison with the model of fig. 2 the behaviour of a dough cylinder and of a strip of washed gluten* are shown in figs. 3 and 4 by plotting simply the load against the length. In these experiments, when

* Gluten test-pieces are prepared in the following way: A small ball of dough is kneaded continuously under the tap until all the starch has been washed away, and the wash water is clear. The mass of wet gluten, which consists largely of protein and water, is given coherence by further kneading, and a small strip of approximately rectangular cross-section is cut with a razor. When extended on the trough of the extensimeter, this loses its angles, and recovers to the shape of a slightly irregular cylinder whose diameter can be measured with a fair degree of accuracy.

the cylinder had been extended as far as was considered safe, the synchronous motor was reversed and the cylinder was allowed to contract at the same rate as it had been extended.

It will be seen that for the greater part of their length the loading branches of the curves bend upwards. This is the behaviour to be expected of a system of springs irregularly assembled, some of which do not come under stress at all until a certain amount of elongation has taken place. Such a curvature would also appear where the springs are approaching their greatest possible extension.

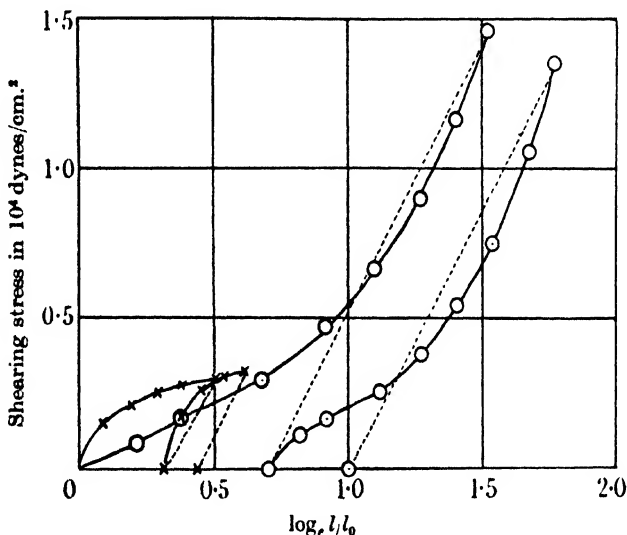


FIG. 5—○ Control. × HCl treated.

It is evident from the close resemblance between figs. 3 and 4 that the elastic structure in the dough is the gluten. It is, therefore, not unreasonable, in view of the work of Astbury (1933) on protein fibres, to suggest that the branched protein chains of the gluten are the springs securely fastened together, and that the insecure links are made by the electrostatic attraction between oppositely charged groups of neighbouring molecules.

Support for this view was obtained from an experiment in which hydrochloric acid was added to a dough slightly in excess of the amount needed to convert all the COO^- groups into COOH . The maximum shearing stress that this dough would stand without tearing was about 0.35×10^4 dynes/cm.², whereas the control dough made up to the same moisture content but without the addition of acid easily withstood 1.5×10^4 dynes/cm.². The curves are shown in fig. 5.

The effect of 10 min. drastic remixing of a dough is shown in fig. 6, from which it will be seen that this treatment caused the cylinder to flow out under a relatively low stress. The normal condition was, however, largely restored by allowing the remixed dough to stand for 2 hr.

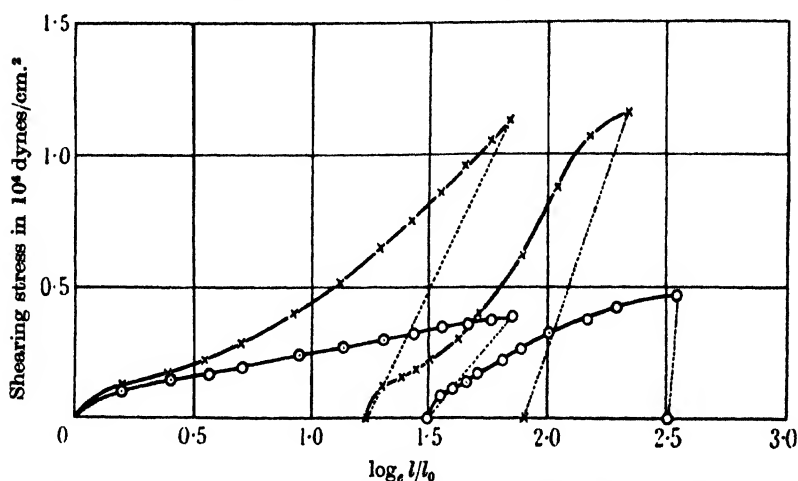


FIG. 6—○ Immediately after drastic remixing. × After resting further 2 hr.

Hence it was evident that a considerable time is required for the elastic structure in a dough to become linked up. In order to investigate this point further, cylinders were prepared from a freshly made dough and kept on the mercury bath under small waterproof covers for varying lengths of time before being tested. The cylinders were extended for 5 min. by which time they had been stretched by $\log_e l/l_0 = 1.28$, and the stress was recorded. The stress was then released and $\log_e l/l_0$ again determined after 5 min. free contraction. They were again extended, this time until rupture occurred, and their greatest length was measured. Judged by the length at rupture

TABLE I

Age (hours)	...	0.33	1.0	2.0	2.75	4.5	7.5
First extension							
Shearing stress for $\log_e l/l_0 = 1.28$ in 10^4 dynes/cm. ²		1.22	0.71	0.61	0.65	0.62	0.58
$\log_e l/l_0$ after recovery		0.69	0.78	0.77	0.77	0.87	0.91
Second extension							
Length at rupture (cm.)		20	33	29	34	32	32

there is little further build-up of structure after 1 hr., but it will be seen that the flow during the first extension, as measured by $\log_e l/l_0$ after recovery,

continues to increase up to 7 hr. notwithstanding the fall in shearing stress. This may be due to water released by syneresis of the gluten diluting the starch paste, and thereby reducing its viscosity (*vide infra*).

The Influence of the Starch—In comparing the curves for dough and gluten shown in figs. 3 and 4, one is struck by their very close similarity. In fact, apart from a tendency for the loops to be wider in the case of the dough, there is only one noticeable difference. At the lowest strains, the stress rises very steeply in the case of dough to what almost is a yield value, whereas with gluten there is considerable deformation even at the lowest stresses. The only difference between gluten and dough is that the latter contains starch, whereas in the former this ingredient has been removed. Starch paste has an anomalous viscosity, i.e. one that is higher the lower the shearing stress, and so would be expected to influence the behaviour of the dough more at low than at high stresses.

Some additional experiments in which equilibrium was approached first by a simple recovery from a small load, and secondly after having momentarily overloaded the sample, showed that the loops in figs. 3 and 4 are mainly due to true hysteresis and not to any great extent to elastic after-effect: they would have appeared even if the cycles had been carried out very much more slowly.

It was also found that under very small loads applied in the range where no permanent deformation occurs, dough shows an elastic fatigue on repeated loading, but that it slowly recovers on resting. This is evidently a type of thixotropic behaviour.

SUMMARY

Experiments are described which support the view that in a flour dough the gluten forms an elastic network which dominates the mechanical behaviour. It appears that when a cylinder of dough is first stretched some of the links in the network are ruptured since it will not return to its original length. Enough remain unbroken, however, for a continuity of structure to be preserved until the cylinder has been extended to five or six times its original length. The "work hardening" of dough is thus accounted for. The elastic network does not establish itself at once, but continues to build up for some time after the dough is mixed. Its strength is greatly reduced by drastic remixing of the dough but is largely recovered on further standing. The addition of hydrochloric acid in slight excess of the acid binding capacity destroys the strength of the network. This shows that the electro-

static attraction between oppositely charged groups in neighbouring molecules is an important factor in the strength of the gluten network.

The upward bend of the reloading curve up to the point where flow (i.e. the rupture of further links) occurs is probably mainly due to the irregularity of assembly of the elastic members, but may also indicate that individual chains are approaching the limit to which they can be extended.

Evidence has been obtained that the starch paste penetrating the gluten network has a "yield value", in consequence of which there is elastic hysteresis even when the cycle is carried out slowly enough to avoid elastic after-effect.

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Über die grundlegenden mechanischen Eigenschaften des Mehnteiges.

Von R. K. Schofield und G. W. Scott Blair.¹⁾
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Zusammenfassung früherer Arbeiten.

Zum Verständnis der Prozesse, die bei der Brotherstellung eine Rolle spielen, muß man ein klares Bild von den mechanischen Vorgängen besitzen, die bei der Einwirkung von Zug- und Scherkräften auf Brotteig auftreten. Eine Reihe von Forschern haben dieses Problem von der technischen Seite aus betrachtet und Eigenschaften gemessen, die aber noch nicht in die Grundkomponenten aufgelöst werden konnten. Selbst für rein industrielle Zwecke bringt das Zurückführen der mechanischen Eigenschaften auf die

Grundeinheiten offensichtliche Vorteile mit sich. Für ein wissenschaftliches Verständnis der beteiligten Prozesse ist dagegen ein solches Vorgehen unerlässlich. Bei der Dehnung von Mehlteig sind die auftretenden Deformationen teilweise elastischer und teilweise plastischer Natur. Hieraus ergeben sich zwei physikalische Eigenschaften grundlegender Bedeutung: Die Viskosität und der Schermodul. Da die Poisson'sche Zahl von Teig 0,5 beträgt, besteht keine Notwendigkeit, den Young'schen Modul als getrennten Faktor zu behandeln, da dieser dem dreifachen des Schermoduls entspricht, und der Volumenmodul eine Größenordnung höher liegt.

¹⁾ Übersetzt von K. Hoffmann (Leipzig).

Abgesehen von der grundlegenden Arbeit von Kosutány [1907] (1) sind keine weiteren experimentellen Untersuchungen dieser Grundeigenschaften vor dem Jahre 1932 angestellt worden, als die Verfasser der vorliegenden Arbeit die erste einer Reihe von Untersuchungen über dieses Thema veröffentlichten. Kosutány streckte Teigzylinder in konstantem Verhältnis und maß die dazu erforderliche Kraft. Er konnte seine Ergebnisse mit einigem Erfolg mit der Backfähigkeit vergleichen, unterließ es jedoch, die wichtige Unterscheidung zwischen reversiblen und irreversiblen Deformationen einzuführen.

Die Verfasser veröffentlichten eine Reihe von Arbeiten (2), in deren erster sie zeigen konnten, daß die Dauer der Krafteinwirkung im Verhältnis zu der entsprechenden Relaxationszeit (Viskosität/Schermodul, nach Maxwell) maßgebend ist für das Verhältnis von elastischer (reversibler) und plastischer (irreversibler) Deformation. Es wurde der Verlauf der Kräfte an Teigstücken, die in gestreckter Lage gehalten wurden, für eine Anzahl von Kräften verfolgt. (τ_r) und (η) fallen mit steigender Kraft. Es wurde gefunden, daß der Teig eine Eigenschaft ähnlich der Streckhärtung von Metallen zeigt, als deren Ergebnis (τ_r) und (η) für eine gegebene Kraft von der Gesamtdeformation abhängen.

In der zweiten Arbeit (3) wurde eine andere Methode angewandt, um die Effekte der Strukturviskosität [Fallen von (η) mit steigender Kraft; vgl. Ostwald (4)], von denen der Streckhärtung zu trennen. Teigzylinder wurden lotrecht in einer geschlossenen Röhre aufgehängt und konnten sich unter der Einwirkung der Schwerkraft ausdehnen. Die Deformationen wurden gemessen, indem eine vorher auf die Probestücke gezeichnete Millimeterskala nach dem Versuch durch Aufpressen auf Papier übertragen wurde. Diese Abzüge wurden Rheogramme genannt und ließen die Deformationen deutlich erkennen. In der dritten Arbeit (5) wurde gezeigt, daß der Teig Eigenschaften der elastischen Nachwirkungen und Hysterese zeigt und daß infolgedessen ein zusätzliches Glied in die Maxwell'sche Gleichung eingeführt werden muß, die dann folgendermaßen lautet.

$$\frac{de}{dt} = \frac{1}{\eta} \left(\frac{dS}{dt} - \frac{da}{dt} \right) + \frac{1}{\eta} S \quad (1)$$

wobei $\frac{de}{dt}$ = zeitliche Änderung der Streckung,
 $\frac{dS}{dt}$ = zeitliche Änderung der Scherkraft
und a = einen Faktor, der die elastische

Nachwirkung berücksichtigt, bedeuten.

In der vierten Arbeit (6) werden Experimente mit sehr hohen Beträgen der Dehnung beschrieben und ein mechanisches Modell zur Veranschaulichung des mechanischen Verhaltens von Mehlteig vorgeschlagen. Es wurde gezeigt, daß das Phänomen des Fließens in Teigen grundverschieden von dem des gewöhnlichen viskosen Fließens sein dürfte und auf Reißen von schwachen Bindungen in der Proteinstruktur des Teiges zurückzuführen ist. Ein Vergleich mit gewaschenem Kleber zeigte, daß mit Ausnahme von schwachen Kräften die Stärke allein kaum dieses Verhalten verursachen konnte. Es bildet sich im stehenden Teig eine Proteinstruktur, die vorübergehend durch heftiges Schütteln und dauernd durch Zugabe von Salzsäure in einer Menge, die die säurebindende Kapazität des Glutens (Klebers) schwach übersteigt, zerstört werden kann.

Eine Zusammenfassung dieser drei Arbeiten wurde für deutsche Leser in einer weiteren Veröffentlichung gegeben (7).

In der ersten von drei Arbeiten von Halton und Scott Blair (8) wurde die Elastizität des Teiges näher untersucht, und es konnte gezeigt werden, daß diese Eigenschaft von hoher Viskosität und niedrigem Schermodul, d. i. hoher Relaxationszeit abhängt. Hefe, die in üblichen Mengen zugesetzt wird, hat erstaunlich wenig Einfluß auf die beschriebenen Effekte. Eine sehr sorgfältige Ausführung der Versuche ist notwendig, um reproduzierbare Ergebnisse zu erhalten. In der zweiten Arbeit (9) wurde die Neigung des Teiges, wenn er gezogen ist, zu zerreißen („Kürze“), untersucht, und es konnte gezeigt werden, daß diese Eigenschaft in einer allgemeinen Beziehung zur Strukturviskosität steht. [Die nähere Natur dieser Verwandtschaft wurde ebenfalls durch die Verfasser diskutiert (10).] Die dritte Arbeit (11) ist eine mehr technische Zusammenfassung der ersten beiden, die durch weitere Experimente vervollständigt wurde.

Scott Blair und Potel (12) beschreiben Experimente mit dem Chopin-Extensimeter (13), in denen der Bruch und die Deformation beim Einblasen von Luft in Teig gemessen wird, und zeigen, daß diese Faktoren komplexe Funktionen der Viskosität und des Schermoduls sind.

Bohn und Bailey (14, 15) haben eine Beziehung zwischen bestimmten Werten, die nach der Schofield-Scott Blair-Methode erhalten wurden, und der Fähigkeit des Teiges, längerem Schütteln zu widerstehen, gegeben.

Wolarowitsch und Samarina (16) weisen darauf hin, daß die Buckingham-Reiner-Gleichung, die diese Autoren auf das Fließen von Lehmpasten (17) anwenden, auch für Mehlteige, die in einem rotierenden Viskosimeter untersucht wurden, gilt.

Die vorliegende Arbeit hat das Ziel, zum Teil eine Zusammenfassung früherer Untersuchungen zugunsten deutscher Leser zu geben, anderseits einige weitere neue Experimente über das Verhalten von Mehlteig unter Einwirkung kleinerer Kräfte zu beschreiben.

Experimente mit kleinen Kräften.

Wenn eine Zugkraft auf einen frisch präparierten Teigzylinder ohne vorherige Ausdehnung ausgeübt wird, gibt es kein wahres elastisches Gebiet, sondern ein Fließen tritt bereits bei sehr kleinen Dehnungen auf. Läßt man dagegen einen Zylinder nach einer Dehnung um ungefähr 15 Proz. wieder bis zum Erreichen konstanter Länge zusammenschrumpfen, so bewirken nachfolgende kleine Ausdehnungen rein elastische Deformationen. Man kann also sagen, daß die vorhergehende Dehnung eine Härtung des Zylinders verursacht. Experimente in dem Gebiet kleiner Kräfte wurden mit einem Spezial-Extensimeter, das Fig. 1 zeigt, ausgeführt.

Zwei hohle Metallbehälter werden so angeordnet, daß das Wasser eines Thermostaten durch sie fließen kann. Der untere wurde mit einer Wachsschicht ausgekleidet, und der obere, der als Deckel diente, wurde mit Filz bedeckt. Der Teig schwamm auf Quecksilber in dem unteren Behälter und war durch einzelne ungesponnene Seidenfäden mit zwei Hebelarmen verbunden. Das System für die Messung der Aus-

dehnung wurde gerade eben stabil, aber schwach gegen den Teig geneigt, ausgeführt. Die Stellung des Zeigers gibt ein Maß für die Dehnung des Teiges. Der ganze Behälter konnte zur Einstellung des Nullpunktes durch Drehung einer Welle in horizontaler Richtung verschoben werden. Der Ausschlag des Zeigers für die Messung der Kraft war durch Arretierungen so begrenzt, daß er nur kleine Veränderungen seiner Lage machen konnte.

Der von dem Teig ausgeübte Zug wurde durch eine Meßkette, aus deren Stellung die Spannung abgelesen werden konnte, kompensiert. Die Stelle, bei der der Zeiger gerade zwischen der Arretierung frei schwingen konnte, konnte durch das Aufblitzen einer Lampe bestimmt werden. Diese Lampe wurde durch einen am Zeiger befestigten Quecksilberkontakt eingeschaltet. Eine Zugkraft konnte ausgeübt werden entweder durch Aufsetzen von Gewichten auf den freien Arm des Dehnungshebels oder indem man den Zeiger mittels eines seidenen Fadens mit einer von einem Synchronmotor betriebenen Welle verband. Beide Hebelsysteme wurden außerordentlich leicht ausgeführt, ihre Herstellung erfolgte aus dünnen Stahldrähten, die an Rasierklingen angelötet wurden. Da Stärkekpasten bekanntlich eine gewisse Steifheit zeigen, schien es wahrscheinlich, daß auch im elastischen Gebiet nach dem Abschalten der Kraft eine kleine dauernde Dehnung bestehen blieb. Dies würde eine Zerlegung der Hystereseerscheinung, wie es in einer früheren Arbeit (5) beschrieben wurde, in zwei Teile mit sich bringen. Der eine ist dann darauf zurückzuführen, daß zur Ausbildung der elastischen Eigenschaften verschieden lange Zeiträume erforderlich sind, und der andere beruht auf wahrer Hysterese und ist unabhängig von der Zeit.

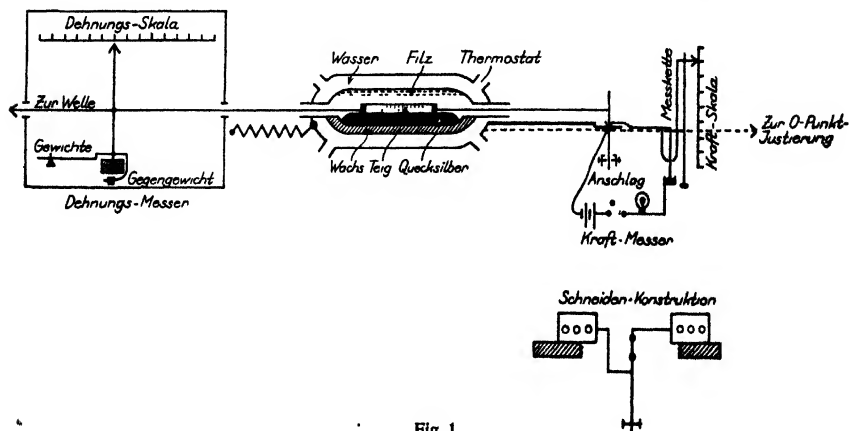


Fig. 1

Um dies genauer zu prüfen, wurde ein gehärteter Probezylinder der Einwirkung einer Last α ($= 0,316$ g) 10 Minuten lang ausgesetzt, konnte sich darauf 10 Minuten lang erholen, und wurde dann wieder mit einem Gewicht $\alpha + \beta$ ($= 0,316 + 0,285$ g) zunächst 5 Minuten lang belastet. Darauf wurde das Gewicht β entfernt, und die Probe 10 Minuten nur mit α belastet, danach auch α entfernt, und die Erholung weitere 10 Minuten lang verfolgt. Dieser Vorgang wurde wiederholt, bis durch die thixotropische Verfestigung die Deformationen unbedeutend klein geworden sind. In Fig. 2 sind die Skalenablesungen, die die Länge des Zylinders anzeigen (in willkürlichen Einheiten) gegen die Zeit aufgetragen. Die Moduln wurden für jedes Zusammenschrumpfen ausgerechnet und unter der ersten Kurve eingezeichnet. Die Moduln steigen während der ganzen Versuchsdauer an, aber es ist klar, daß ein ausgeprägter Unterschied zwischen den mit steigender und fallender Kraft erhaltenen Werten bestehen muß. Das zeigt ein einfacher Vergleich: Wird eine Feder in Stärkepaste zuerst ausgedehnt und kann sich dann zusammenziehen, so wird ihre endgültige Länge größer sein, als wenn der Vorgang umgekehrt verläuft. Die Proteinketten werden sich nun ähnlich verhalten.

Aus den obigen Versuchen geht hervor, daß das Verhalten eines Teigzylinders, der unter Einwirkung kleiner Kräfte gedehnt und zusammengezogen wird, durch die Gegenwart mehrerer Faktoren kompliziert wird. Um dies näher zu untersuchen, wurde ein geeignet gehärteter Teigzylinder durch Anwendung einer konstanten Belastung ent-

sprechend einer anfänglichen Scherkraft von 155 dyn/qcm gestreckt. Nach 30 Sek. wurde die Dehnung abgelesen und die Last entfernt. Nach einer Erholungsdauer von 30 Sek. wurde eine neue Ablesung gemacht, und dieses Spiel verschiedene Male wiederholt. Darauf wurde der Zylinder für 20 Min. in Ruhe belassen, und dann der Prozeß wiederholt. Sodann wurde eine zweite Pause von einer Stunde eingeschaltet, bevor die letzte Reihe von Ablesungen aufgenommen wurde. Die Moduln für das Zusammenschrumpfen wurden ausgerechnet und in Fig. 3 gegen die Zahl der Lastwechsel aufgetragen. Im gleichen Diagramm sind die angenäherten, nicht

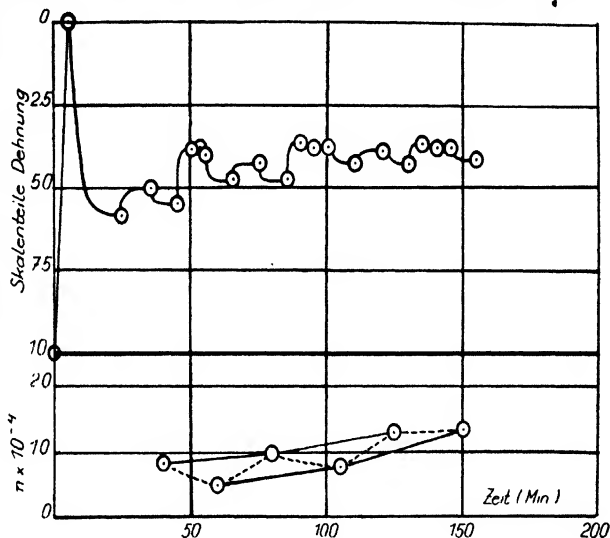


Fig. 2

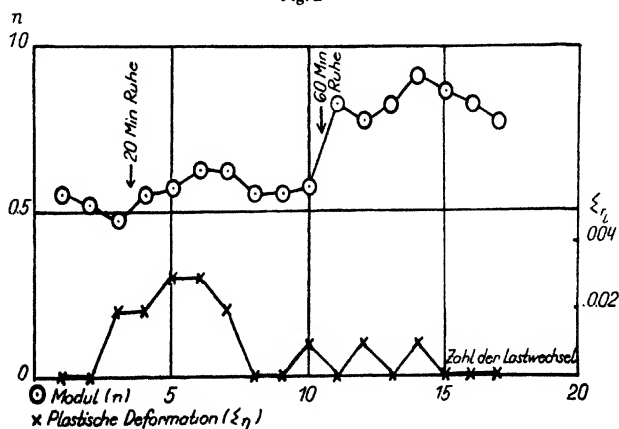


Fig. 3

reversiblen Deformationen in Einheiten der Dehnung ($\Sigma\eta$) eingezeichnet. Es zeigt sich, daß, solange kein Fließen auftritt, die Moduln mit zunehmender Dauer der Einwirkung von Streckung und Schrumpfung abnehmen. Dies ist eine Art von elastischer Ermüdung. Daß man ihn aber auch als eine Art Thixotropieeffekt ansehen kann, zeigt das starke Anwachsen des Moduls beim Stehenlassen des Probezylinders. Dieser Prozeß wird weiterhin durch die Eigenschaft der Streckhärtung kompliziert, da bereits beim geringsten Fließen der Modul stark zu steigen scheint. Dies mag zum Teil auf einem Ansteigen der kritischen Fließgrenze beruhen, andererseits muß man noch daran denken, daß auch die Viskosität des Stärkekontinuums größer geworden ist (innere Viskosität), so daß Messungen der Streckung nach einer gewissen Zeit kein genaues Maß für den Modul mehr ergeben, da der Betrag des elastischen Nachwirkungseffektes abgenommen hat.

Halton hat (unveröffentlichte Ergebnisse) gefunden, daß die Kurve, in der die Viskosität gegen den Modul aufgetragen ist, für ein bestimmtes Mehl bei Einwirkung einer konstanten, ziemlich kleinen Kraft den gleichen Verlauf zeigt, gleichgültig, ob die Änderung dieser Eigenschaften durch Änderung des Wassergehaltes oder verschiedenem Alter des Teiges verursacht wird. Diese Tatsache legt die Vermutung nahe, daß der Alterungsvorgang des Teiges auf einer allmählichen Verdünnung der Stärkepaste durch die vom Protein abgegebene synärethische Flüssigkeit beruht. Diese äußert eine größere wasserbindende Kraft als die Stärke, ist jedoch nicht in der Lage, das anfangs aufgenommene Wasser auf die Dauer zu behalten. Wenn diese Annahme richtig ist, so sollten die Einflüsse des Alters und des Wassergehaltes ausgesprochener bei kleinen Spannungen,

wo die Stärkeanteile an den gemessenen Effekten relativ groß sind, zu beobachten sein.

Einfluß des Feuchtigkeitsgehaltes auf Teig bei kleinen Kräften.

Es wurden Zylinder auf zwei verschiedene und konstante Geschwindigkeiten gestreckt. Eine Gesamtdehnung von 0,177 wurde bei der schnellen Streckung in 57 Sek., bei der langsamen in 275 Sekunden erhalten. Wurden neun Zehntel der Gesamtdeformation erreicht, so wurde die Belastung, die dann nahezu stetig verlief, abgelesen. Wurde die volle Dehnung erreicht, so wurde die Zugkraft abgeschaltet, und der Probe Zeit zur vollständigen Erholung gelassen.

Diese Experimente wurden an einer Reihe von verschiedenen Teigen mit verschiedenem Feuchtigkeitsgehalt ausgeführt, und die Ergebnisse — Kraft gegen reversible Dehnung — in Fig. 4 dargestellt. Es zeigt sich, daß oberhalb eines bestimmten Feuchtigkeitsgehaltes der Betrag der reversiblen Dehnung beinahe unabhängig von der Feuchtigkeitsmenge ist, sobald der Probe genügend Zeit zur Erholung zur Verfügung steht, noch hat eine Variierung der Dehngeschwindigkeit im Verhältnis 1:5 einen größeren Einfluß darauf. In trockenen Teigen ist das Ausmaß der reversiblen Dehnung beachtenswert klein.

Berücksichtigt man diese Ergebnisse, so sieht man leicht ein, daß die großen Änderungen im Modul und der Viskosität, die in den Untersuchungen von Halton und Scott Blair über die Beziehungen zwischen diesen bei Anwendung kleiner Kräfte gemessenen (8, 9) Eigenschaften und der Backfähigkeit eine wichtige Rolle spielen, nicht in dem Maße auftreten, sobald man zu größeren Kräften übergeht. So zeigen Experimente (6), daß der Modul von Teig in der Tat nur ein kleines Anwachsen während des Alters

(mit Ausnahme der ersten 1—2 Stunden) zeigt, und die Viskosität kaum in nennenswertem Maße abnimmt, während Halton und Scott Blair (8) finden, daß die Verringerung dieser beiden Eigenschaften, sobald bei relativ kleinen Kräften gemessen wird, beträchtliche Werte annimmt.

Berechnung von (η) und (n).

In einer früheren Arbeit der Verfasser wurde

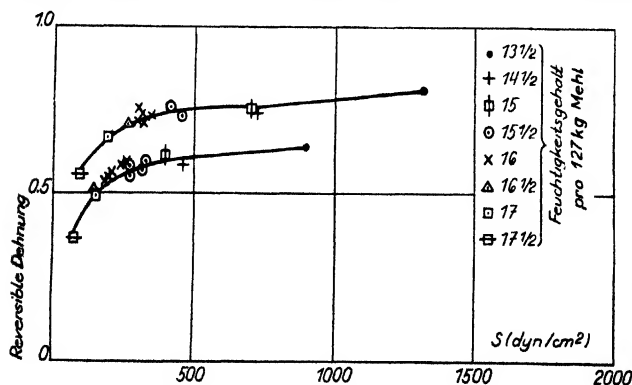


Fig. 4

die hier bereits angeführte Differentialgleichung (Gleichung 1, S. 149) dazu benutzt, um die (η) und (n) aus den experimentellen Beobachtungen zu errechnen. Später haben Halton und Scott Blair mit mittleren Werten dieser Eigenschaften, die man erhält, wenn man die Gleichung (1) integriert, gearbeitet, und es wird in der vorliegenden Arbeit die gleiche Methode angewandt, um die Moduln und Viskositäten auszurechnen. Hierfür ist es erforderlich, die experimentellen Bedingungen so zu wählen, daß der Ausdruck:

$$\int \frac{da}{dt} \cdot dt = 0$$

wird. In der früheren Mitteilung (5) wurde gezeigt, daß sich (a) dem Werte 0 beliebig nähert, wenn S entweder 0 ist oder einen konstanten Betrag annimmt. Dementsprechend verschwindet der Wert des Integrales, sobald der Zylinder eine gegebene Zeit mit konstanter Kraft ausgedehnt wird, und sich dann elastisch ohne Belastung zusammenziehen kann. Auf diese Weise kann man die Gesamtdeformation am Ende der Dehnungsperiode als aus zwei Teilen zusammengesetzt ansehen, einer der rein elastisch ist und während der zweiten Periode wieder verschwindet, und dem übrigbleibenden Rest, der bestehen bleibt. Einen mittleren Wert für η erhält man nun, wenn die konstante der während der ersten Periode angewandten Kraft durch den Betrag der irreversiblen Deformation dividiert und mit der Zeitdauer der Krafteinwirkung multipliziert wird. Einen mitt-

leren Wert für n erhält man durch Division der Kraft durch die reversible Dehnung.

Diese Anschauung verlangt, daß der Betrag der irreversiblen Dehnung nur durch die Anwendung einer äußeren Kraft verändert wird, dagegen nicht durch die inneren Kräfte, die die elastische Nachwirkung hervorrufen, beeinflusst werden kann. Mit anderen Worten: Es darf keine „plastische Nachwirkung“, die auf Gleiten der Proteinketten gegeneinander während der Periode des Zusammenschrumpfens beruht, geben. Mit Rücksicht auf die Schwierigkeiten, einen gegebenen Teig reproduzierbar zu gestalten, konnte dieser Punkt nicht leicht geklärt werden, und es bedurfte einer großen Zahl von Experimenten, bis man das Ergebnis, daß eine solche Erscheinung der plastischen Nachwirkung nicht existiert, als einigermaßen gesichert betrachten konnte. Zwei Teigzylinder wurden so ähnlich als möglich hergestellt und der eine (a) auf dem Extensimeter bei kleiner konstanter Kraft bis zu einer bestimmten Dehnung gestreckt und ihm dann Zeit zum Schrumpfen gegeben. Der zweite (b) wurde zunächst in kleinerem Maße gedehnt, konnte sich dann erholen und wurde darauf zum vollen Betrage gestreckt, worauf er erneut zusammenschrumpfen konnte. Die Ergebnisse sind in Fig. 5 aufgezeichnet, in der die Dehnung gegen die Kraft aufgetragen wurde. Obgleich die Beträge der endgültigen reversiblen Dehnung sich nur wenig unterscheiden, zeigen die Ergebnisse einer großen

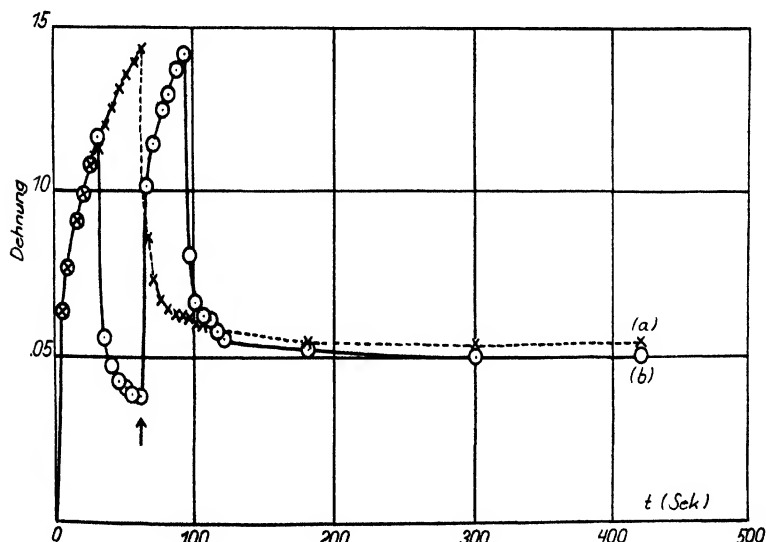


Fig. 5

Zahl von Experimenten, daß die Abweichungen willkürlich in beiden Richtungen verteilt sind, und beweisen damit, daß keine plastische Nachwirkung besteht. Der Pfeil in der Figur bezeichnet die Gesamtzeit, während der (b) tatsächlich unter Spannung stand. Die Zeit paßt gut zu der, die im Falle (a) der gleichen Dehnung entspricht.

Zusammenfassung.

Es wird eine Übersicht über Arbeiten, die sich mit der Viskosität und dem Schermodul von Brotteig befassen, gegeben und gezeigt, daß nur durch Untersuchung der grundlegenden Eigenschaften ein Fortschritt im Verständnis, sei es nun im Verhalten des Teiges als rein physikalisches Problem oder im Hinblick auf die Beziehung der physikalischen Eigenschaften zu seiner Backfähigkeit, erreicht werden kann.

Es hat den Anschein, als ob die Stärkepaste, die das Netzwerk des Glutens durchsetzt, eine kritische Fließgrenze hat, woraus das Bestehen einer elastischen Hysterese auch dann folgt, selbst wenn die Belastungswechsel so langsam vorgenommen werden, daß eine elastische Nachwirkung vermieden wird. Außerdem wurde gefunden, daß das Zusammenbrechen des Gelgerüsts, das sich beim Stehen bildet und sich in einer elastischen Ermüdung äußert, thixotropische Ursachen hat. Der Effekt wird durch eine Zunahme des Elastizitätsmoduls wahrscheinlich dank der Neubildung von Vernetzungen im Glutennetzwerk zum Teil verdeckt.

Infolge der Einflüsse der Stärke sind die mechanischen Eigenschaften des Teiges durch Alterung und Feuchtigkeitsgehalt stärker geändert, wenn sie bei Einwirkung kleinerer als größerer Kräfte gemessen werden. Die reversible Dehnung von Teigzylindern, die in gegebener Zeit nur um etwa 20 Proz. gestreckt wurden, variiert

sowohl mit dem Alter als auch mit dem Feuchtigkeitsgehalt des Teiges und mit der Natur des Mehles.

Es wurde gefunden, daß während des elastischen Zusammenschrumpfens kein plastisches Fließen auftritt. Das Auftreten von „plastischer Nachwirkung“ würde die zur Berechnung der Viskosität und des Schermoduls angewandte Methode beeinträchtigen.

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A FURTHER NOTE ON THE ARRANGEMENT OF VARIETY TRIALS: QUASI-LATIN SQUARES

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I. INTRODUCTION

A NEW method of arranging agricultural field trials involving a large number of varieties has recently been described by the author⁽³⁾ under the name of "pseudo-factorial arrangements", but better, perhaps, termed *quasi-factorial* arrangements. Such arrangements are likely to be of utility in any experimental work in which a large number of treatments have to be compared and in which the experimental material falls into small groups of closely similar units.

In a quasi-factorial varietal trial the varieties are divided into sets in two or more ways, the varieties of each set being arranged in one or more randomized blocks. The block size can thus be kept small even when the number of varieties is very large, and all use of controls is avoided. In a two-dimensional quasi-factorial arrangement of 81 varieties, for example, the varieties, after being numbered at random from 1 to 81, are divided into a group of 9 sets consisting of varieties 1, 9, 10, 18, 19, 27, ..., 73, 81, and a similar group of 9 sets consisting of varieties (1, 10, 19, ..., 73), (2, 11, 20, ..., 74), ..., (9, 18, 27, ..., 81), each of these sets being arranged at random in one or more blocks of 9 plots. It will be seen that these sets form the rows and columns of a diagrammatic square of the varietal numbers.

In a square two-dimensional quasi-factorial arrangement further divisions of the varieties into groups of sets are also possible, each group being such that every set of the group includes one and only one variety from each set of every other group. If in the case of p^2 varieties $p + 1$ such groups are formed, a completely orthogonal system results. Since every two treatments then occur together once and once only in a block, we arrive at a special case of the type of arrangement described in (1) and there called an arrangement in symmetrical incomplete randomized blocks.

In the present paper a further extension of the quasi-factorial principle is described, whereby differences associated with two different groupings of the experimental material can be simultaneously eliminated. This type of arrangement may be called an arrangement in *quasi-Latin squares**, from the analogy with ordinary Latin square arrangements. In varietal trials in quasi-Latin squares each complete replication of the varieties is arranged in the field in a square pattern, all differences between both rows and columns being eliminated from the varietal comparisons, just as they are in an ordinary Latin square.

Quasi-Latin squares are less flexible than ordinary quasi-factorial arrangements, since

* Or alternatively an arrangement in *lattice squares* (see note on nomenclature at the end of the paper).

the number of varieties or treatments must be a perfect square (or a perfect cube) and certain perfect squares, in particular 36, are inadmissible. The designs are likely to be of considerable practical utility, however, because if the two sources of variation associated with two different groupings of the experimental material are of equal magnitude, the simultaneous elimination of both sources is more than twice as effective in reducing experimental error as is the elimination of one source only. The effectiveness of Latin square arrangements in agriculture, for example, has long been recognized.

II. STRUCTURE OF QUASI-LATIN SQUARES

If the number of varieties is a perfect square (equal to p^2 say), then for certain values of p it is possible to divide the varieties into $p + 1$ orthogonal groups of p sets (each set containing p varieties), i.e. in such a manner that each set of any one group of sets contains one and only one variety from each set of any other group of sets. The three groups of sets corresponding to the rows, columns and letters of a Latin square fulfil the conditions of orthogonality. The $p + 1$ groups can therefore be formed from a completely orthogonal set of $p - 1$ squares. Such completely orthogonal sets are known to exist for values of p which are prime numbers, and also for $p = 4, 8$ and 9 (1). No such set exists for $p = 6$ (2).

If $p + 1$ such groups of sets exist, then the $p^2 - 1$ degrees of freedom representing differences between varieties partition into $p + 1$ groups of $p - 1$ degrees of freedom, each group corresponding to the $p - 1$ contrasts between the p sets of the corresponding group of sets. Each replication may therefore be arranged in the field in the form of a square of which the rows correspond to one group of sets and the columns to a second, so that in every replication the degrees of freedom corresponding to two groups of sets will be confounded with row or column differences. If p is odd and there are $\frac{1}{2}(p + 1)$ replications, each group of sets may be confounded once and once only in this manner, and in this case equal information will be obtained on every degree of freedom, and therefore every varietal comparison will be made with equal accuracy.

When p equals 5, for instance, the four squares given in Table I form a completely orthogonal set.

Table I. *Orthogonal set of 5×5 squares*

Square 1	Square 2	Square 3	Square 4
a b c d e	a b c d e	a b c d e	a b c d e
e a b c d	d e a b c	c d e a b	b c d e a
d e a b c	b c d e a	e a b c d	c d e a b
c d e a b	e a b c d	b c d e a	d e a b c
b c d e a	c d e a b	d e a b c	e a b c d

(The law of formation, which is the same for all prime numbers, should be obvious from inspection of this table. For $p = 4, 8$ and 9 , orthogonal sets are given in (1).)

If there are 25 varieties and these are numbered 1-25 at random, the first row of each square may be taken to represent the varieties 1-5, and so on. So long as every group of sets is confounded equally it is immaterial which are confounded in each replication. If we confound the groups corresponding to rows and columns in the first, those corresponding to squares

1 and 3 in the second and those corresponding to squares 2 and 4 in the third replication, then the first replication must be arranged on the ground in a square pattern so that the varieties 1-5 come in one row (not necessarily the first), the varieties 6-10 in another and so on. At the same time varieties 1, 6, 11, 16, 21 must come in one column, varieties 2, 7, 12, 17, 22 in another and so on. We must in fact randomize the rows and columns of the square:

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25

This randomization process is that adopted in ordinary Latin squares in order to ensure an unbiased estimate of error.

In the second replication the varieties corresponding to the *a*'s of the first square, namely varieties 1, 7, 13, 19, 25, must come in one row, and so on. At the same time the varieties corresponding to the *a*'s of the third square, namely, 1, 9, 12, 20, 23, must come in one column, and so on. We must therefore randomize the rows and columns of the square:

		Square 3					
		<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	
Square 1	<i>a</i>	1	13	25	7	19	
	<i>b</i>	20	2	14	21	8	
	<i>c</i>	9	16	3	15	22	
	<i>d</i>	23	10	17	4	11	
	<i>e</i>	12	24	6	18	5	

the structure of this square being given by the marginal letters.

Similarly the third replication is obtained by the randomization of the rows and columns of the square:

		Square 4					
		<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	
Square 2	<i>a</i>	1	15	24	8	17	
	<i>b</i>	18	2	11	25	9	
	<i>c</i>	10	19	3	12	21	
	<i>d</i>	22	6	20	4	13	
	<i>e</i>	14	23	7	16	5	

The statistical analysis of balanced arrangements such as this is very simple. It is first necessary to calculate for each variety a quantity pQ , equal to p times the sum of the yields of all the plots of that variety, less the totals, $p+1$ in number, of every row and every column in which that variety occurs. The varietal differences, in terms of the yield of a single plot, are then given by the differences of the quantities

$$\frac{2}{p-1} Q \quad \text{or} \quad \frac{2}{p(p-1)} pQ.$$

A quantity equal to $\frac{2p}{p-1}$ times the mean yield should be added to each in order that their mean should equal the general mean.

The standard error of each *difference* is

$$\sqrt{2} \times \sqrt{\frac{2}{p-1}} \times \text{the standard error of a single plot.}$$

The sum of squares due to varieties in the analysis of variance is

$$\frac{2}{p-1} \text{dev}^2 Q \quad \text{or} \quad \frac{2}{p^2(p-1)} \text{dev}^2 pQ,$$

where $\text{dev}^2 Q$ indicates the sum of the squares of the deviations of Q from their own mean. The first and last of these formulæ are given in two forms, the first form being most easily remembered, the second being that required for computation.

The remainder of the analysis of variance proceeds in the ordinary manner, items for rows, columns and squares being included to allow for the fertility differences eliminated by the design.

It should be noted that each of the above expressions can be derived from the parallel formula applicable to an experiment with the same number $\frac{1}{2}(p+1)$ of replications arranged in ordinary randomized blocks of p^2 plots, by writing $\frac{2}{p-1}$ for $\frac{2}{p+1}$ and replacing the sum of the yields of each variety by the corresponding Q .

It follows from this that the efficiency factor of the arrangement is

$$\frac{p-1}{p+1}.$$

This factor represents the loss of efficiency that would result if there were no gain in accuracy by the elimination of fertility differences between the rows or between the columns.

In this section we have only considered arrangements in which every set of degrees of freedom is confounded equally. Such arrangements may be called *balanced* quasi-Latin squares. This balance is analogous to the balance of designs in symmetrical incomplete randomized blocks (4). Sets of squares which lack this balance are also feasible, and are of interest in such cases as 8×8 , which requires nine replications for complete balance, but in which nearly complete balance can be attained with four replications.

Such arrangements lose very little in efficiency through the slight lack of balance, the efficiency factors in the case of 8×8 squares being $\frac{7}{9}$ and $\frac{27}{36}$ or 0.778 and 0.771 respectively. The computations are somewhat more complicated, owing to the fact that the set of row and column totals entering into a single Q is no longer completely balanced for varieties, and an additional term must therefore be introduced to restore this balance.

III. NUMERICAL EXAMPLE

We will take as an example the uniformity trial on oranges reported by Parker and Batchelor, the results of which have already been used to illustrate quasi-factorial arrangements in randomized blocks. The mean yields of the first six years for the whole trial are given in Table V of (3). The yields (less 100) of the first fifteen plots of each of the first five blocks are reproduced in Table II. The table also shows a superimposed arrangement of twenty-five varieties, indicated by the small numbers, which is the result of randomizing the rows and columns of the three squares given in the preceding section. The orientation of each square has also been allotted at random.

Table II. *Yields (less 100) and arrangement of varieties*

Plot	Block					Total
	<i>M</i>	<i>L</i>	<i>K</i>	<i>J</i>	<i>I</i>	
2	-13 ²³	3 ²¹	-4 ²⁵	3 ²²	13 ²¹	2
4	-5 ⁸	0 ⁸	19 ¹⁰	4 ⁷	20 ⁹	44
6	11 ¹⁸	10 ¹⁶	9 ²⁰	-1 ¹⁷	40 ¹⁹	69
8	-21 ³	12 ¹	-1 ⁵	15 ²	29 ⁴	34
10	-2 ¹³	13 ¹¹	16 ¹⁵	10 ¹²	22 ¹⁴	59
Total	-30	44	39	31	124	208
12	2 ²⁶	9 ⁶	22 ¹⁴	28 ¹⁷	13 ³	74
14	-7 ⁷	26 ¹⁸	37 ²¹	28 ⁴	8 ¹⁵	92
16	0 ¹⁹	16 ⁵	29 ⁸	19 ¹¹	13 ²²	77
18	-7 ¹³	15 ²⁴	12 ²	19 ¹⁰	20 ¹⁶	59
20	-2 ¹	16 ¹²	27 ²⁰	35 ²³	25 ⁹	101
Total	-14	82	127	129	79	403
22	-2 ¹⁰	38 ¹⁹	32 ³	37 ²¹	38 ¹²	143
24	3 ²³	(18) ⁶	21 ²⁰	19 ¹³	15 ⁴	76
26	-3 ¹⁸	21 ²	4 ¹¹	10 ⁹	17 ²⁵	49
28	19 ¹	19 ¹⁵	29 ²⁴	32 ¹⁷	29 ⁸	128
30	8 ¹⁴	31 ²³	48 ⁷	30 ⁵	52 ¹⁶	169
Total	25	127	134	128	151	565

The yield of the plot 24 of the block *L* was missing from the original records. The omission of this row entirely would be unduly unfavourable to the Latin square design, since the whole row is low-yielding, and the row has therefore been retained, with the value 118 for the missing yield, calculated from the row and column values of the third square.

The quantities $5Q$ are shown in Table III (negative signs being omitted). Thus, for example,

$$5Q_1 = 5 \times 12 - 44 - 34 + 5 \times (-2) + 14 - 101 + 5 \times 19 - 25 - 128 = -173.$$

Table III

Varities	Values of $-5Q$				
1-5	173	187	314	246	304
6-10	282	231	232	250	259
11-15	312	247	228	318	309
16-20	161	264	117	136	261
21-25	151	195	233	244	226

The adjusted yields of the varieties are shown in Table IV. Thus, for example, the adjusted yield of variety 1 equals

$$\frac{10}{4}(\text{mean yield}) + \frac{2}{5.4} 5Q_1 = 139.2 - \frac{1}{10}(173) = 121.9.$$

ARRANGEMENT OF VARIETY TRIALS

Table IV

Varieties	Adjusted varietal means				
1-5	121.9	120.5	107.8	114.6	108.8
6-10	111.0	116.1	116.0	114.2	113.3
11-15	108.0	114.5	116.4	107.4	108.3
16-20	123.1	112.8	127.5	125.6	113.1
21-25	124.1	119.7	115.9	114.8	116.6

The analysis of variance is shown in Table V. The sum of squares for varieties is given by the sum of the squares of the deviations of the values of Table III divided by 25, 4/2 or 50.

Table V. *Analysis of variance*

	D.F.	Sums of squares	Mean square
Squares	2	2556.24	1278.12
Rows	12	2696.08	224.67
Columns	12	7108.08	592.34
Varieties	24	1566.64	65.28
Error	24	1381.28	57.55
Total	74	15308.32	

The mean square for varieties is 65.28, and that for error is 57.55. Nothing has been deducted for the missing plot, since the yield of this was determined independently of the varietal arrangement. The mean squares for varieties and error are approximately equal, as they should be.

The standard error assignable to each of the adjusted values of Table IV is given by

$$\sqrt{\left(\frac{2}{4} \times 57.55\right)} = 5.36.$$

IV. RELATIVE EFFICIENCY OF VARIOUS ARRANGEMENTS

The higher efficiency of Latin squares compared with randomized blocks is likely in general to more than compensate for the lower efficiency factors of quasi-Latin squares. It will be recalled that the efficiency factors for two-dimensional quasi-factorial arrangements (p^2 varieties) are $\frac{p+1}{p+3}$, $\frac{p+1}{p+2}$ and $\frac{p}{p+1}$ according as two, three or $p+1$ groupings are used. The numerical values of these factors, and of the factor $\frac{p-1}{p+1}$ for quasi-Latin squares, are shown in Table VI.

Table VI. *Efficiency factors*

No. of varieties p	16	25	49	64	81	121	169
Minimum no. of replications for quasi-Latin squares	4	5	7	8	9	11	13
	5	3	4	9	5	6	7
Quasi-Latin squares	0.6	0.667	0.75	0.778	0.8	0.833	0.857
Quasi-factorials in blocks:							
Two groupings	0.714	0.75	0.8	0.818	0.833	0.857	0.875
Three groupings	0.769	0.8	0.842	0.857	0.870	0.889	0.903
$p+1$ groupings	0.8	0.833	0.875	0.889	0.9	0.917	0.929

All values of p from $p = 4$ to $p = 13$ have been included for which a completely orthogonal set of squares is known to exist. The even values $p = 4$ and $p = 8$ will require $p + 1$, i.e. five and nine replications in order to obtain a balanced arrangement. In many cases, of course, some multiple of the minimum number of replications will be required to attain the desired accuracy.

It will be noted that for quasi-factorial arrangements in blocks the balanced arrangement with $p + 1$ groupings requires $p + 1$ replications. It is an additional advantage of quasi-Latin squares that when p is odd balance is attained with half the number of replications required for quasi-factorial arrangements in randomized blocks. The attainment of balance has three advantages. The efficiency is maximized, the standard errors of all varietal comparisons are the same, and the computations are simplified.

The efficiency of the various arrangements in the example of the preceding section may now be considered. Table VII gives the residual mean squares after eliminating squares only, squares and columns or rows, and squares, columns and rows. These are the mean error mean squares that will be obtained in arrangements in randomized blocks of 25, in arrangements in randomized blocks of 5, and in 5×5 Latin squares respectively.

Table VII. *Residual mean squares*

	D.F.	Mean square	Information
Blocks of 25	71	179.61	1
Blocks of 5 (columns)	59	95.66	1.88
Blocks of 5 (rows)	59	170.44	1.05
5×5 Latin squares	47	62.72	2.86

In each case one degree of freedom has been deducted to allow for the missing plot. This procedure is approximate except in the case of the Latin squares.

This table provides an excellent illustration of the power of the Latin square design in eliminating fertility differences. Although in this case the elimination of row effects alone would scarcely have reduced the residual variance, their elimination subsequent to that of columns has effected a substantial reduction. In general it is easy to see that, if the variance due to rows is equal to that due to columns, the elimination of both rows and columns will bring about a relative reduction of the residual variance of more than twice that due to the elimination of either alone.

Multiplying the relative amounts of information per plot of Table VII by the efficiency factors of Table VI, we obtain the relative efficiencies shown in Table VIII. Thus in this particular example the use of quasi-Latin squares instead of randomized blocks containing all the varieties almost doubles the information obtained.

In order to illustrate the increase in precision resulting from the use of quasi-Latin squares, an arrangement of twenty-five varieties in randomized blocks of twenty-five plots (corresponding to the squares already used) was also superimposed on the variety trial. The varietal means so obtained are shown in Table IX. Their variability is easily seen to be

considerably greater than that of the adjusted varietal means of Table IV, the ranges in the two cases being from 99.7 to 131.3, and from 107.4 to 127.5. The two distributions of values

Table VIII. *Relative efficiencies of various arrangements*

	Efficiency in chosen example	Efficiency when there are no fertility differences to eliminate
Randomized blocks of 25 plots	100	100
Quasi-factorial arrangements in blocks of 5 plots:		
Two groupings	140.8	75
Three groupings	150.2	80
Six groupings	156.5	83.3
Quasi-Latin squares	190.9	66.7

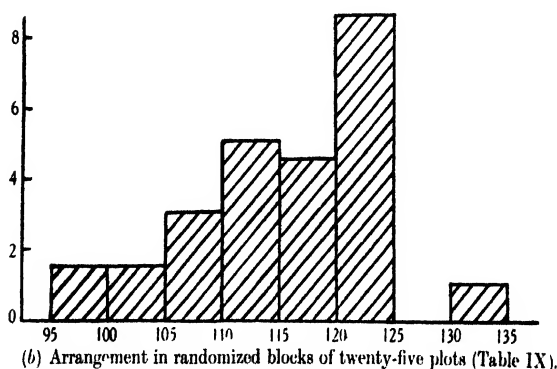
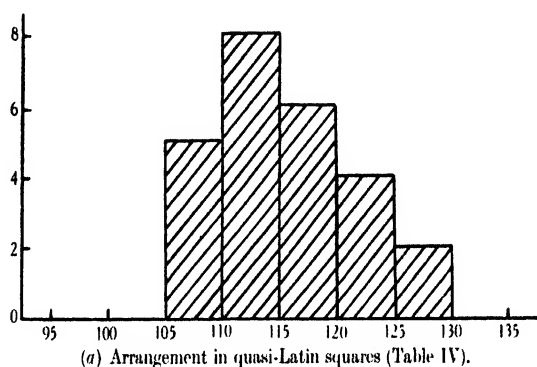


Fig. 1. Distributions of the twenty-five varietal means from Table IV and Table IX.

are also compared graphically in Fig. 1. (Both sets of values happen to give a fair representation of the amount of variation that would be obtained on the average in this trial from the two types of arrangement.)

Table IX. *Varietal means from arrangements in randomized blocks of twenty-five plots*

104.3	118.7	120.0	112.0	111.7
114.0	122.7	109.3	124.3	108.7
107.7	122.3	119.7	99.7	115.3
100.0	121.3	121.0	122.0	112.3
131.3	117.3	120.7	121.0	114.7

In this example quasi-Latin squares have also proved markedly more efficient than quasi-factorial arrangements in randomized blocks, though the example cannot be regarded as particularly favourable to the Latin square arrangement, since blocks account for the greater part of the fertility irregularities. In general it may be doubted whether quasi-factorial arrangements in randomized blocks are likely to result in any great gain in efficiency when the number of varieties is as small as twenty-five. It would appear, however, that even with this small number of varieties quasi-Latin squares are likely to be very effective. It has been found, for example, that in the Rothamsted experiments and experiments at associated centres from 1927 to 1934 the error variance of 5×5 Latin squares was reduced on the average in the ratio of 2.49:1 from what it would have been if the experiments had been completely randomized. This, multiplied by the efficiency factor $\frac{2}{3}$, gives an average increase of 66 per cent in the information when 5×5 quasi-Latin squares instead of randomized blocks of twenty-five plots are used for varietal trials involving twenty-five varieties.

Latin squares are, of course, only suited to certain types of variety trial. With crops that are sown by drill the practical requirements of drilling may necessitate long narrow plots, and preclude the use of a Latin square design. In such crops as fruit, however, this consideration does not hold, and even with long narrow plots the additional restrictions of a Latin square are often strikingly effective in reducing the error variance.

V. THE USE OF QUASI-LATIN SQUARES IN THREE-DIMENSIONAL QUASI-FACTORIAL DESIGNS

If a number p^3 of varieties is appropriately divided into three groups of p^2 sets of p varieties each, and each of these sets is arranged in one or more randomized blocks, a three-dimensional quasi-factorial arrangement results. The division into the three groups of sets may be effected by setting out the varieties at random in a cube and taking the sets lying on lines parallel to the edges of the cube. The analysis of such arrangements was discussed in (3),

where it was shown that the efficiency factor was
$$\frac{2(p^2 + p + 1)}{2p^2 + 5p + 11}.$$

In certain cases quasi-Latin squares can be used as the basis of an arrangement of this type, for if the varieties be divided into p sets of p^2 varieties in two ways, orthogonal to one another, the members of each set can be compared by means of a set of quasi-Latin squares. The appropriate division can be effected by taking the sets lying on planes parallel to two of the faces of a random cube of the varieties. If sufficient replications are available, the

group of sets corresponding to planes parallel to the third face may also be taken, but there is little further gain in efficiency. The efficiency factors for the arrangements using two and three groupings are

$$\frac{p-1}{p+1} \cdot \frac{p^2+p+1}{p^2+p+3} \quad \text{and} \quad \frac{p-1}{p+1} \cdot \frac{p^2+p+1}{p^2+p+2\frac{1}{2}}$$

respectively.

Arrangements of this type compare favourably with ordinary three-dimensional quasi-factorial arrangements in randomized blocks, for the advantages of Latin square design are obtained without any great reduction in the efficiency factor below that of the randomized block arrangements. Table X gives the numerical values of the efficiency factors for the various types of arrangement for $p = 4, 5$ and 7 .

Table X. *Efficiency factors for three-dimensional arrangements*

No. of varieties	64	125	343
p	4	5	7
Minimum no. of replications, two groupings	10	6	8
Quasi-Latin squares:			
Two groupings	0.548	0.626	0.725
Three groupings	0.560	0.636	0.731
Randomized blocks	0.607	0.721	0.792

Whether these arrangements are likely to be more efficient than the equivalent two-dimensional arrangements in quasi-Latin squares depends on the additional reduction in variance that results from the reduction in size of the Latin squares.

The estimation of the varietal differences is best carried out in two stages. The quantities Q are first calculated for the various sets of quasi-Latin squares, and from them estimates of the varietal means are obtained for each grouping. These estimates can then be set out in three-way tables (one for each grouping) and an adjusted table prepared in a similar manner to that adopted in quasi-factorial designs in randomized blocks. With a similar factorial notation to that previously used in (3) the adjusted varietal means t_{uvw} are given by

$$t_{uvw} = \frac{1}{2}(x_{uvw} + y_{uvw} - \bar{x}_u. + \bar{x}_v. + \bar{y}_u. - \bar{y}_v.),$$

when the two groups of sets are formed by holding u and v constant respectively. This formula is almost the same as that for two-dimensional quasi-factorial arrangements in randomized blocks on p. 433 of (3). In the case of three groupings the formula for t_{uvw} is identical with that given on p. 438 for two-dimensional quasi-factorial arrangements in three groups of sets.

The comparisons between the t_{uvw} are not all of exactly the same precision, the variances in the case of two groupings of the differences of two t 's of varieties occurring together in two, one or no sets of quasi-Latin squares being

$$\frac{p+1}{p-1}, \quad \frac{p+1}{p-1} \cdot \frac{p^2+1}{p^2}, \quad \frac{p+1}{p-1} \cdot \frac{p^2+2}{p^2}$$

respectively times the corresponding variance when there are no restrictions and the error

mean square is unchanged. Thus even with two groupings the variation in precision is so small that the mean error variance given by the efficiency factor will suffice for all practical purposes (except possibly for varieties having both sets of quasi-Latin squares in common). In the case of three groupings the variation in precision will be even smaller.

The analysis of variance involves no new principle, the procedure to be followed being a combination of the ordinary procedure for quasi-Latin squares, and the procedure for quasi-factorial arrangements in randomized blocks.

VI. THE USE OF THE QUASI-LATIN SQUARES PRINCIPLE IN FACTORIAL DESIGN

In varietal trials we are equally interested in comparisons between every pair of varieties, and consequently the aim of the design is to confound all comparisons equally frequently. In experiments involving several factors, however, we are usually less concerned with the high order interactions than with the effects of single factors and interactions between two factors only. We may consequently be prepared to sacrifice some or all of the information on one or more of the high order interactions, provided that the efficiency of the remaining comparisons is thereby increased.

If the quasi-Latin square type of design is used for an experiment involving several factors, therefore, the condition that every set of degrees of freedom is confounded equally may be dispensed with. Instead we may confine the confounding to sets of degrees of freedom representing high order interactions, keeping the main effects free from confounding.

If, for example, instead of sixty-four varieties we have an experiment including all combinations of eight varieties and two levels of each of the three standard fertilizers, i.e. an $8 \times 2 \times 2 \times 2$ factorial design, the treatment degrees of freedom will partition into

Varieties	7
Fertilizers	7
Fertilizers \times varieties	49

If we take the rows and columns of a completely orthogonal set of 8×8 squares to represent varieties and fertilizers respectively, seven groups of sets of the varietal and treatment combinations will be determined by these seven squares. Contrasts between sets of the same group will correspond to seven of the 49 degrees of freedom for interaction, the whole seven sets accounting for the whole 49 degrees of freedom. Each set of seven degrees of freedom will be found to be of the form

$$\begin{array}{lll} V_1.N & V_4.N.P & \\ V_2.P & V_5.N.K & V_7.N.P.K \\ V_3.K & V_6.P.K & \end{array}$$

where V_1, V_2, \dots, V_7 are seven orthogonal degrees of freedom for varieties of the form

$$V_1 = v_1 + v_2 + v_3 + v_4 - v_5 - v_6 - v_7 - v_8,$$

etc., V_4 being given by the "interaction" of V_1 and V_3 , etc.

In each replication two such sets may be confounded, one with the rows and one with

the columns, so that if there are three replications $\frac{3}{8}$ of the relative information will be obtained on 42 of the 49 interaction degrees of freedom, and full information on the remainder.

A variant of this design is that in which there are only four varieties, so that we have a $4 \times 2 \times 2 \times 2$ factorial design. A single square will then give two complete replications, and one set of three degrees of freedom can be confounded with rows and another with columns.

Sets of the type

Rows	Columns
$V_1.N.P$	$V_1.N.K$
$V_2.N.K$	$V_2.P.K$
$V_3.P.K$	$V_3.N.P$

are possible. In this case a single replication will sacrifice all the information on these degrees of freedom, the analysis being of the form:

Rows	7
Columns	7
Varieties	3
Fertilizers	7
Unconfounded interactions	15
Error	24
Total	63

This design is derivable from the $8 \times 2 \times 2 \times 2$ design by using duplicate varieties.

A simpler example of the same type of design is provided by the arrangement of a $2 \times 2 \times 2$ design in two or more 4×4 quasi-Latin squares, confounding two interaction degrees of freedom, one with rows and one with columns, in each square. The following two squares will confound the interactions shown:

(1) np nk pk	(1) k np npk
npk k p n	nk pk n p
n p k npk	p n pk nk
pk nk np (1)	npk np k (1)
Rows: $N.P.K$	Rows: $N.P$
Columns: $P.K$	Columns: $N.K$

Thus with two replications we might sacrifice half the information on each of the interaction degrees of freedom. Alternatively all information on $N.P.K$ and $P.K$ might be sacrificed, so as to obtain full precision on $N.P$ and $N.K$.

Similar designs are possible with factors at three levels. In a $3 \times 3 \times 3$ experiment with three replications, for instance, we may confound one pair of degrees of freedom for the interaction between the three factors with the rows, and a second pair with the columns.

Partial confounding within the limits of a single square is also possible, provided of course that the square comprises more than a single replication. Thus in a 2^5 design in a single 8×8 square eight of the ten three-factor interactions, and four of the five four-factor interactions, may be partially confounded, one half the relative information being obtained on each.

In all such designs it can easily be shown that the randomization of rows and of columns provides an unbiased estimate of error. Since, however, the original squares are of a rather special type, the designs even after randomization possess certain systematic elements which are at first sight disconcerting. Thus in the $3 \times 3 \times 3$ design the three levels of any one factor (or numbers representing the contrasts corresponding to the unconfounded interactions) can be brought into the pattern given by Fig. 2 (a), and a typical pattern after randomization is that of Fig. 2 (b):

(a)									(b)								
0	0	0	1	1	1	2	2	2	2	1	0	0	0	1	2	1	2
0	0	0	1	1	1	2	2	2	1	0	2	2	2	0	1	0	1
0	0	0	1	1	1	2	2	2	0	2	1	1	1	2	0	2	0
1	1	1	2	2	2	0	0	0	0	2	1	1	1	2	0	2	0
1	1	1	2	2	2	0	0	0	1	0	2	2	2	0	1	0	1
1	1	1	2	2	2	0	0	0	0	2	1	1	1	2	0	2	0
2	2	2	0	0	0	1	1	1	2	1	0	0	0	1	2	1	2
2	2	2	0	0	0	1	1	1	2	1	0	0	0	1	2	1	2
2	2	2	0	0	0	1	1	1	1	0	2	2	2	0	1	0	1

Fig. 2

It is not, perhaps, very likely that the z distribution will be appreciably disturbed, but it would be satisfactory to have confirmation of this point by developing the distribution by randomization over a series of uniformity trials.

VII. SUMMARY

The principles of quasi-factorial design are extended so as to enable trials involving a number of varieties or treatments which is a perfect square (not 6^2 or some other numbers, however) to be so arranged that differences associated with two groupings of the experimental material, such as the rows and columns of an agricultural field trial, are simultaneously eliminated from the varietal comparisons.

As a numerical example a quasi-Latin square design for 25 varieties is superimposed on the uniformity trial on oranges which was used in a previous paper to illustrate quasi-factorial designs in randomized blocks. A gain in efficiency over an arrangement in ordinary randomized blocks of 91 per cent resulted, the corresponding gain in a quasi-factorial design in randomized blocks (two groupings) being 41 per cent.

Various other possible applications of the quasi-Latin square principle are briefly discussed.

NOTE ON NOMENCLATURE

Since the above paper was written, I have provided an alternative and shorter nomenclature for quasi- (or pseudo-) factorial designs, using the term *lattice*, which enables the various types of design to be described very concisely. The following table of equivalents will make clear the sense in which the word is used.

Two-dimensional quasi-factorial designs in randomized blocks:	
In two equal groups of sets	Lattice or square lattice
In two unequal groups of sets	$p \times q$ lattice
In three (equal) groups of sets forming a Latin square	Triple lattice
In $p + 1$ (equal) groups of sets	Balanced lattice
Three-dimensional quasi-factorial designs in randomized blocks:	
In three equal groups of sets	Cubic lattice or three-dimensional lattice
In three unequal groups of sets	$p \times q \times r$ lattice
Balanced set of quasi-Latin squares	Lattice squares
Three-dimensional quasi-factorial design in quasi-Latin squares	Cubic lattice in lattice squares

The terms *quasi-factorial* and *quasi-Latin square* may be usefully retained as general descriptive terms. In particular the term *quasi-Latin square* appears specially appropriate for the factorial designs outlined in section VI. Various designs of this latter type have been developed in detail in (5).

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THE χ^2 DISTRIBUTION FOR THE BINOMIAL AND POISSON SERIES, WITH SMALL EXPECTATIONS

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§ 1. INTRODUCTION

ONE of the more common uses of the χ^2 distribution is to compare the variance in a sample of N from the binomial series distribution $(p+q)^n$, with the expected population variance $n\hat{p}\hat{q}$, where \hat{p} and \hat{q} are estimated from the sample. A single member of the binomial series distribution (i.e. a sample of one) is taken to mean the number of successes in n independent trials, in each of which the chance of success is p . A sample of N members may be written x_1, x_2, \dots, x_N , where any x is an integer between 0 and n . For large expectations it is known, cf. (1), that

$$\Sigma \frac{(x-\bar{x})^2}{n\hat{p}\hat{q}} = \Sigma \frac{n(x-\bar{x})^2}{\bar{x}(n-\bar{x})}$$

is distributed as χ^2 with $(N-1)$ degrees of freedom.

The quantity $\frac{\Sigma n(x-\bar{x})^2}{\bar{x}(n-\bar{x})}$ has been called the index of dispersion for the binomial series.

The following is an example of its use in tests of significance. In a study of the distribution of diseased plants in a field, it is of interest to know whether diseased plants tend to congregate in patches, an effect which might occur, for instance, if the disease were liable to spread from one diseased plant to its neighbours. This point may be studied by dividing the field into small areas, each containing say twenty plants, and calculating the total number x of diseased plants in each area. If diseased plants are grouped together there will be more areas with many diseased plants or with very few diseased plants than would occur in random sampling from the binomial series. The index of dispersion will thus be larger than expectation and the χ^2 test may be used to detect the grouping of diseased plants.

I recently recommended (2) the use of this test in the study of the distribution of diseased plants in a field or greenhouse in cases in which the expectations were likely to be small, perhaps as low as 1 or 2. It is not known in these cases how satisfactory a representation of the distribution of

$$\Sigma \frac{(x-\bar{x})^2}{n\hat{p}\hat{q}}$$

can be obtained from the ordinary χ^2 distribution, and this paper was originally intended as a discussion, with some numerical examples, of the agreement between the true and the χ^2 distribution in this case. Some notes have also been added on the general question

of the use of χ^2 as a test of discrepancies between observation and hypothesis with small expectations, and, while no essentially new points have arisen, the importance of the subject perhaps justifies a brief discussion.

§ 2. SOME EXAMPLES OF THE EXACT DISTRIBUTION OF χ^2

The distribution of χ_s^2 , defined as $\frac{\sum n(x - \bar{x})^2}{\bar{x}(n - \bar{x})}$ for the binomial series, depends on three variables, the size N of the sample, the index n of the binomial, and the expectation $m = np$ of success. Of these m and N are the most important, and for fixed m and N the distribution changes slowly and regularly as n increases from 1 to ∞ , in which case the distribution becomes the Poisson series with mean m . This property was used by Yates(3) in his discussion of the 2×2 contingency table and appears to hold quite generally. When N is large, m and n remaining small, the distribution of χ_s^2 , like that of the tabular χ^2 , tends to normality, though not with quite the same mean or variance as χ^2 . The normal approximation to χ_s^2 will be given in the next section and can be used in tests of significance when appropriate.

When N and m are small, the exact distribution of χ^2 may be found in any particular case without much computation. It is first necessary to find the probability of obtaining any given configuration.

Let x_1, x_2, \dots, x_N be the numbers of successes out of n in that order in a sample of N values of the binomial series distribution, where

$$T = x_1 + x_2 + \dots + x_N$$

The probability of obtaining such a sample is

$$P = \prod_{i=1}^N \binom{n}{x_i} p^{x_i} q^{n-x_i},$$

$$= \frac{(n!)^N p^T q^{nN-T}}{\prod x_i! (n-x_i)!},$$

and $\sum P = 1$, taken over all possible samples.

In estimating p we make the population total coincide with the sample total T , and the frequency distribution of χ^2 is accordingly to be taken only over the set of samples with total T . To find the probabilities in this set it is necessary to divide P by $\sum P$ taken over the members of the set. But this is the probability of obtaining T successes in a single trial of the binomial $(p+q)^{nN}$, and is therefore

$$\frac{(nN)!}{T! (nN-T)!} p^T q^{nN-T}.$$

Hence the probability of drawing the sample x_1, x_2, \dots, x_N in that order out of the set with total T is

$$P' = \frac{(n!)^N T! (Nn-T)!}{(Nn)! \prod x_i! (n-x_i)!}.$$

If the sample is regarded as representing a $2 \times N$ contingency table, with marginal totals T , $Nn - T$, and N n 's, the probability is seen to be the product of the factorials of the marginal totals divided by the product of the factorials of the grand total and the individual cell numbers. This result holds generally for contingency tables, as was pointed out by Yates (3), p. 233).

The corresponding result for a sample of N from the Poisson series is obtained by making n tend to infinity. This gives

$$P' = \frac{T!}{\prod x_i!}.$$

In practice what is wanted is the probability of the configuration (x_1, \dots, x_N) , irrespective of the order in which they come. If x_1, \dots, x_N were all distinct, this would be $N! P'$, and if there are a_1 0's, a_2 1's, etc., the probability is

$$\frac{N!}{\prod a_i!} P'.$$

Thus the probability of obtaining a_1 x_1 's, a_2 x_2 's, etc., in a sample of N is

$$\frac{N! (n!)^N T! (Nn - T)!}{(Nn)!} \times \frac{1}{\prod \{ (x_i)! (n - x_i)! \}^{a_i} (a_i)!}.$$

In any particular case in which the exact distribution of χ_s^2 is required, only the second part is variable. The practical procedure is first to enumerate all possible configurations. For each configuration there is a probability and a value of χ_s^2 . By forming a table of the values of $\{(x_i)! (n - x_i)!\}$ for $x_i = 0, 1, 2, \dots, n$ and using logs the work may be reduced to a minimum.

In cases where the expectation $m (= np)$ is not too small, so that the agreement between the χ_s^2 and the χ^2 distribution is fairly close, the chief source of discrepancy is that the former is a discontinuous distribution and the latter continuous. The true values of χ_s^2 are, however, always evenly spaced, apart from values with zero probability, since the values of $\Sigma (x - \bar{x})^2$ change by 2's when arranged in increasing order, and a correction for continuity may easily be made by taking χ^2 less by half an interval than the value which is being tested. For low values of m , however, other sources of disturbance have become important, and the continuity correction does not necessarily improve the agreement in the region in which the probability lies between 0.1 and 0.01. For instance, the χ_s^2 distribution has a finite range, while that of χ^2 is from 0 to ∞ , and this introduces a tendency for χ^2 , when corrected for continuity, to overestimate the true probabilities near the end of the tail. At the other end of the distribution, which is of less practical importance, the χ^2 distribution seems always to overestimate the probability of obtaining so good an agreement by chance.

The cases $m = 2$, $N = 4$ are shown in Table I for $n = 8$ and $n = \infty$. There are only 15 distinct configurations, each of which gives a separate value of χ_s^2 . The probabilities shown are those of obtaining a value of χ_s^2 at least as great as that given.

Table I

$n=8$				$n=\infty$ (Poisson)			
χ_s^2	P	P' tabular χ^2	P' cor- rected for continuity	χ_s^2	P	P' tabular χ^2	P' cor- rected for continuity
0	1.000	1.000	1.000	0	1.000	1.000	1.000
1.3	0.942	0.722	0.881	1	0.962	0.801	0.919
2.6	0.541	0.446	0.573	2	0.654	0.573	0.682
4.000	0.426	0.262	0.343	3	0.551	0.392	0.476
5.3	0.183	0.149	0.198	4	0.295	0.262	0.321
6.6	0.120	0.0834	0.112	5	0.218	0.172	0.213
8.000	0.0489	0.0461	0.0620	6	0.116	0.112	0.139
9.3	0.0380	0.0252	0.0341	7	0.0951	0.0720	0.0898
10.6	0.0094	0.0137	0.0186	8	0.0336	0.0461	0.0576
12.000	0.0066	0.0074	0.0101	9	0.0272	0.0203	0.0368
14.6	0.0030	0.0021	0.0040	11	0.0169	0.0117	0.0186
16.0	0.0010	0.0011	0.0016	12	0.0067	0.0074	0.0093
22.6	0.0001	0.0001	—	17	0.0015	0.0007	0.0023

The two highest values of χ^2 have been omitted in both distributions. In the region below 0.1, the ordinary distribution shows no consistent tendency either to overestimate or underestimate, while the corrected distribution generally overestimates the probability and gives no better agreement on the whole. The maximum discrepancies in the probabilities in the region used in tests of significance are of the order of 40 per cent; whether this is considered satisfactory will depend on the standard of accuracy required.

Comparing the Poisson distribution with the binomial $n=8$, it will be seen that the interval between successive values of χ_s^2 has shortened by one-third in the former case, while the probabilities of the values themselves are less for the Poisson series than the binomial with low values of χ_s^2 and greater with high values. This appears to be the general type of change in the distribution as n tends to infinity. The agreement between the χ_s^2 and ordinary χ^2 distributions is of the same order in both cases.

Table II below gives a summary of the results obtained in this and four other examples. The maximum percentage discrepancies, both positive and negative, in the probabilities in the range $0.1 \geq p \geq 0.005$ are shown for the uncorrected and the corrected distributions.

Table II

m	N	n	Number of values of χ_s^2	Maximum percentage discrepancy			
				Ordinary χ^2		Corrected χ^2	
				+	—	+	—
3.0	6	6	17	12	21	48	—
2.0	4	8	13	46	34	98	10
2.0	4	∞	13	37	31	71	6
1.8	10	9	27	4	9	33	—
1.8	5	9	14	9	21	44	—
0.9	10	9	17	11	30	70	—

The numbers of values of χ_s^2 given are those which account for all but 0.0001 of the total probability. The examples taken are all extreme cases, the total number of values of χ_s^2 being small, and were all computed without much labour.

For the uncorrected χ^2 distribution the largest absolute percentage deviation is negative in four out of the five cases, indicating that the uncorrected χ^2 distribution tends to underestimate the probability of a discrepancy in this range. The corrected distribution, on the other hand, definitely overestimates; in only one case, $m=2$, $N=4$, was there any underestimation at all. The absolute percentage discrepancies are in all cases considerably larger for the corrected than for the ordinary χ^2 distribution. The agreement between the true and the uncorrected χ^2 distribution is, in fact, surprisingly good; the maximum discrepancies are under 25 per cent except in the cases in which $m=0.9$ or $N=4$.

The conclusion from this very limited examination of the possible range of variation of m , N and n is the reassuring one that the tabular χ^2 distribution will give a satisfactory approximation to the true distribution in the region used in tests of significance, except in those cases in which the number of possible configurations is so small that it is easy to calculate the true distribution. The only exception to this rule seems to be the case in which m and n are small but N is large; this will be dealt with in the next section.

§ 3. THE NORMAL APPROXIMATION TO THE χ_s^2 DISTRIBUTION

The notation used in this section will be that adopted by Fisher⁽⁴⁾. In this k_r is the consistent estimate from the sample of the r th semi-invariant κ_r , and κ ($2^2 1^3$), for instance, is κ_{23} of the joint distribution of k_2 and k_1 . In this notation $\chi_s^2 = \frac{(N-1) k_2}{\kappa_2}$.

When N is large, the distribution of χ_s^2 tends to normality, and tests of significance may be made when the mean and variance are known. If we are sampling from a binomial distribution with known p ,

$$\text{Mean } (\chi_s^2) = (N-1),$$

$$\begin{aligned} V(\chi_s^2) &= \frac{(N-1)^2}{\kappa_2^2} V(k_2) = \frac{(N-1)^2}{\kappa_2^2} \kappa(2^2) \\ &= \frac{(N-1)^2}{\kappa_2^2} \left\{ \frac{2\kappa_2^2}{N-1} + \frac{\kappa_4}{N} \right\} \\ &= 2(N-1) \left\{ 1 + \frac{(N-1)}{2N} \frac{\kappa_4}{\kappa_2^2} \right\} \quad \dots\dots(1) \\ &= 2(N-1) \left\{ 1 + \frac{(N-1)(1-6pq)}{2N npq} \right\} \quad \dots\dots(2). \end{aligned}$$

This is less than the variance $2(N-1)$ of the ordinary χ^2 distribution if $1-6pq < 0$, i.e. if $0.21 \leq p \leq 0.79$. It is the appropriate variance to use in the normal approximation where the value of p in the binomial series is known and does not have to be estimated.

Where the value of p has to be estimated from the sample, as is usually the case, the variance and the mean value of χ_s^2 are required in that array in which the mean of the sample is equal to the true mean of the population. The restriction on the mean would not affect the distribution of χ_s^2 if the latter were independent of the distribution of the mean. This is the case for the normal distribution but not for the binomial series.

The moments of the distribution of χ_s^2 in arrays in which the mean is fixed may be obtained to any order of approximation by a method similar to that used by K. Pearson (5), which is of wide application.

If x and y are two correlated variates referred to the means of their distributions, their moment generating function

$$M = \iint e^{t_1 y + t_2 x} f(xy) dx dy$$

$$= 1 + \frac{t_2^2}{2!} \mu_{20} + \frac{t_1}{1!} \frac{t_2}{1!} \mu_{11} + \frac{t_2^2}{2!} \mu_{02} + \dots \quad \dots\dots(3).$$

Integrating first with respect to y we have

$$M = \int e^{t_2 x} dx g_0(x) \left[1 + g_1(x) t_1 + g_2(x) \frac{t_1^2}{2!} + \dots \right],$$

where $g_1(x)$, $g_2(x)$, ... are the moments of the distribution of y in arrays in which x is fixed. Expanding $e^{t_2 x}$ we get

$$M = \int g_0(x) dx \left[1 + x t_2 + x^2 \frac{t_2^2}{2!} + \dots \right] \left[1 + g_1(x) t_1 + g_2(x) \frac{t_1^2}{2!} + \dots \right] \quad \dots\dots(4).$$

By comparing coefficients of powers of the t 's in (3) and (4) the moments of the functions $g_0(x)$, $g_r(x)$ may be obtained. The general expression is

$$\mu_{rs} = \int x^s g_0(x) g_r(x) dx \quad \dots\dots(5).$$

Approximations will be obtained to $g_1(x)$ and $g_2(x)$ which are polynomials in x . Let $\psi_0(x)$, $\psi_1(x)$, ... be a set of polynomials in x of degree 0, 1, ..., etc., which are orthogonal to $g_0(x)$, i.e. such that

$$\int g_0(x) \psi_r(x) \psi_s(x) dx = 0 \quad \text{if } r \neq s.$$

It is easy to see that if the coefficient of x^r in ψ_r is taken to be unity

$$\psi_0 = 1, \quad \psi_1 = x, \quad \psi_2 = x^2 - \frac{\mu_{03}}{\mu_{02}} x - \mu_{02}.$$

Write

$$g_1(x) = a_0 \psi_0 + a_1 \psi_1 + a_2 \psi_2.$$

Then

$$0 = \int g_0 g_1 \psi_0 dx = a_0 \int g_0 \psi_0^2 dx = a_0.$$

Since we want $g_1(0)$, the value of a_1 need not be found.

For a_2 ,
$$\int g_0 g_1 \left(x^2 - \frac{\mu_{03}}{\mu_{02}} x - \mu_{02} \right) dx = a_2 \int \left(x^2 - \frac{\mu_{03}}{\mu_{02}} x - \mu_{02} \right)^2 dx,$$

i.e.
$$\mu_{12} - \frac{\mu_{03}}{\mu_{02}} \mu_{11} = a_2 \left(\mu_{04} - \frac{\mu_{03}^2}{\mu_{02}} - \mu_{02}^2 \right) = a_2 \mu_{02}^2 (2 + \gamma_2 - \gamma_1^2),$$

where γ_1, γ_2 are Fisher's measures of departure from normality for the distribution of x . If $y = k_2$ and $x = k_1$, and all symbols refer to the original distribution, this becomes

$$\kappa(21^2) - \frac{\kappa(1^3)}{\kappa(1^2)} \kappa(21) = a_2 \kappa(1^2) \left(2 + \frac{\gamma_2 - \gamma_1^2}{N} \right),$$

i.e.
$$\frac{1}{N} \left(\kappa_4 - \frac{\kappa_3^2}{\kappa_2} \right) = a_2 \kappa_2 \left(2 + \frac{\gamma_2 - \gamma_1^2}{N} \right).$$

The deviation of the mean of the array $x = 0$ from the true mean of the whole distribution is to this order of approximation

$$\begin{aligned} a_2 \psi_2(0) &= -\mu_{02} a_2 \\ &= \frac{\kappa_2}{N} \frac{(\gamma_1^2 - \gamma_2)}{\left(2 + \frac{\gamma_2 - \gamma_1^2}{N} \right)}. \end{aligned}$$

Hence the deviation in χ_s^2 is (neglecting terms in $\frac{1}{N^2}$)

$$\frac{1}{2} (\gamma_1^2 - \gamma_2) \left[1 - \frac{1}{N} \left\{ 1 - \frac{\gamma_1^2 - \gamma_2}{2} \right\} \right] \quad \text{.....(6).}$$

For the binomial series,
$$\gamma_1^2 = \frac{(q-p)^2}{npq}, \quad \gamma_2 = \frac{1-6pq}{npq}.$$

$$\gamma_1^2 - \gamma_2 = \frac{2}{n}.$$

$$\therefore \text{Mean} = (N-1) + \frac{1}{n} \left[1 - \frac{1}{N} \left(1 - \frac{1}{n} \right) \right] \quad \text{.....(7).}$$

This formula has been found to give a good approximation with values of N as low as 4. The large sample approximation is

$$(N-1) + \frac{1}{n} \quad \text{.....(8).}$$

For the Poisson series the mean is $(N-1)$ for all values of N .

The same method may be used to obtain an approximation to $g_2(x)$. The expression is rather complicated, if terms of order N^0 are retained, and involves semi-invariants up to κ_6 . The approximation to order N for the variance is

$$V = 2(N-1) \left[1 - \frac{\gamma_1^2 - \gamma_2}{2} \right] \quad \text{.....(9).}$$

This expression could be obtained alternatively by noting that for the bivariate normal distribution the variance of y in arrays in which x is fixed is $\sigma_y^2 (1 - \rho^2)$. For the binomial series the variance is

$$V = 2(N-1) \left(1 - \frac{1}{n} \right) \quad \text{.....(10).}$$

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Thus for the Poisson series, the variance of the χ_s^2 distribution is equal to that of the ordinary χ^2 distribution. This result, however, unlike that for the mean, holds only when N is large.

As an example, the exact χ_s^2 distribution for the case $n=2$, $N=160$, $m=0.4$ was worked out. There are only 24 possible configurations. The formula above gives $M=159.5$, $V=159$, the tabular χ^2 distribution gives $M=159.0$, $V=318$, and the exact values were $M=159.49$, $V=159.28$. The normal approximation, corrected for continuity, gives results correct to

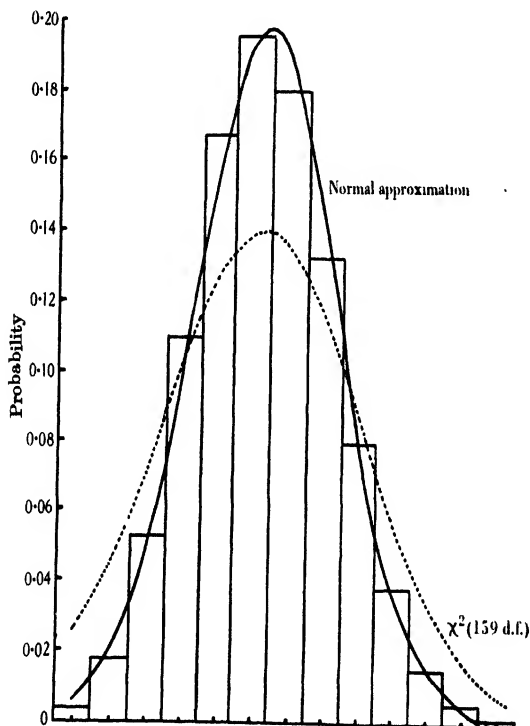


Fig. 1. The histogram shows the χ_s^2 distribution for $m=0.4$, $n=2$, $N=160$.

within 15 per cent in the region used for tests of significance. The three distributions are shown in Fig. 1, the exact χ_s^2 distribution being given as a histogram. It will be seen that the chief cause of discrepancy between the χ_s^2 distribution and the normal approximation is the slight skewness of the former. The tabular χ^2 distribution is a very bad approximation.

For any distribution with finite semi-invariants the distribution of k_2 from a sample of N must tend to normality as N increases, and expressions (1), (6) and (9) may be used to determine the mean and variance, which may be used if the ordinary χ^2 approximation is found to be inapplicable.

§ 4. THE GENERAL χ^2 PROBLEM WITH SMALL EXPECTATIONS

The cases in which the χ^2 distribution is commonly used in tests of significance may be divided into three broad groups, in each of which the theory has been for some time reasonably complete where the expectations involved are large: (1) the comparison of a sample of grouped observations with a hypothetical frequency distribution, which may in the process of fitting be made to agree with the sample in one or more respects; (2) tests of departure from independence in contingency tables; (3) the comparison of the variance calculated from a sample in a discontinuous frequency distribution with the population variance. This is the case which we have been considering.

Little is known about the χ^2 distribution in cases (1) and (2) when the expectations are not large. The only case which has been considered in detail is the 2×2 contingency table, for which Yates⁽³⁾ has shown how to obtain the exact probability distribution. This paper also contains a table which greatly facilitates the use of exact tests of significance with expectations as low as unity; and this particular case, very important in practice, may be regarded as solved.

There are two points involved. It is not known to what extent the true χ^2 distribution deviates from that given in the tables of Fisher and Elderton, and it is not clear that χ^2 , as ordinarily calculated, is the most appropriate general test of discrepancies between observation and hypothesis. Fisher suggested in 1922⁽⁶⁾ that χ^2 derives its validity from the fact that with large expectations it is approximately equal to twice the logarithm (L) of the likelihood of the sample, with its sign changed, and that where this equivalence does not hold, L itself should be used instead of χ^2 . If m is the expectation in any cell and x the value observed,

$$L = Sx \log \frac{x}{m}.$$

The same suggestion was made by Neyman & Pearson⁽⁷⁾ in 1928 as a result of applying their criterion of likelihood to this case. They also calculated⁽⁸⁾ the exact distribution of L and compared it with the tabular χ^2 distribution in samples of 10 from a population divided into three classes with expectations 3, 5 and 2 respectively. They expressed themselves as agreeably surprised with the closeness of the agreement between the L and χ^2 tests in this case. The agreement between the exact and the ordinary χ^2 distributions in the region used in tests of significance was excellent, and the discrepancies between L and χ^2 arose because L arranges the observations in a different order from χ^2 .

In further work on this point, it would seem advisable to concentrate on the distribution of L itself rather than that of χ^2 . For it is just as easy to work out the exact distribution of L as that of χ^2 in particular cases. Further, in contingency tables it is quite common for several distinct configurations to give the same value of χ^2 , whereas in general no two distinct configurations give the same value of L , so that a better representation of the

distribution by a continuous curve should be obtainable for L than for χ^2 . The first approximation to the distribution of $2L = 2Sx \log \frac{x}{m}$ is, of course, the tabular χ^2 distribution. The way in which they appear to diverge with small expectations is shown in Fig. 2, in which the general forms of the χ^2 and ordinary smoothed L distribution are shown.

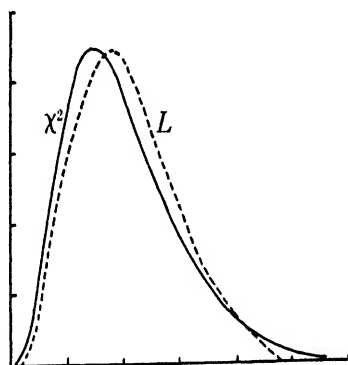


Fig. 2. Comparison of χ^2 and smoothed L distributions.

The two distributions are compared for the Poisson series $m=2$, $N=4$, already considered, in Table III below. The values given in the column headed $P\chi^2$ are obtained by fitting a tabular χ^2 distribution to the distribution of $2L$, just as in Table I we fitted a tabular χ^2 distribution to that of χ_0^2 . The χ^2 distribution has been corrected for continuity as this considerably improves the fit.

Table III

$2L$	$P \geq L$	$P\chi^2$ (corrected for continuity)
6.595	0.1976	0.1084
7.778	0.0951	0.0663
10.412	0.0336	0.0281
11.094	0.0233	0.0131
11.596	0.0169	0.0100
13.185	0.0067	0.0062
16.155	0.0015	0.0020

Except at the end of the scale, the χ^2 distribution considerably underestimates the probability, even after correction for continuity. This appeared in all cases examined and in particular in Neyman & Pearson's example. The agreement is far from satisfactory, though it is not sensibly worse than that between the exact χ_0^2 and the corrected tabular χ^2 in Table I above. It is to be hoped that some frequency distribution will be found which will adequately represent that of L in this region, though no simple adjustment of the tabular χ^2 distribution appears to meet the case.

SUMMARY

Some examples are given of the agreement between the exact and the tabular χ^2 distribution in samples from the binomial and Poisson series with small expectations. The ordinary χ^2 distribution tends slightly to underestimate the probability of discrepancies in the region used in tests of significance, but appears to give a satisfactorily close agreement except in very extreme cases (e.g. with expectations less than unity). Correction for continuity does not improve the agreement.

A method is given for obtaining for any population approximations to any given order for the mean and variance of χ^2 in samples in which the mean of the sample is fixed, and from this the exact normal approximation to the χ^2 distribution for the binomial series is obtained. Except for the Poisson series, this is not the same as the normal approximation to the ordinary χ^2 distribution.

A brief discussion is given of the general problem of testing discrepancies between observation and hypothesis, in which it has been suggested that the likelihood, as defined by Fisher, is more appropriate than χ^2 as a test criterion.

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AN ENUMERATION OF THE CONFOUNDED ARRANGEMENTS IN THE $2 \times 2 \times 2 \dots$ FACTORIAL DESIGNS.

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§ 1. *Introduction.*

THE type of factorial design known as the $2 \times 2 \times 2 \dots (-2^n)$ has been familiar to agricultural experimentalists for a number of years. It is the simplest of the factorial systems, and is useful in exploring the possibilities of new manurial treatments and methods of cultivation, and the interactions which may exist between them. Its application is not confined to agricultural field trials, and there is little doubt that, as the advantages of factorial design become more widely recognized, it will prove to be of use in an increasing number of fields of scientific investigation. Various similar designs have already been utilized, for example, in animal husbandry experiments.

In this system any number, n , of treatment factors, occur at each of two different levels 1, 2, these levels being not necessarily the same for all the factors considered. There are thus 2^n different possible treatment combinations. The material at the experimenter's disposal may be grouped into sets of some power of two on the grounds of similarity. These sets might be blocks of land containing 2, 4, 8 \dots plots as in an agricultural field trial, or litters of pigs in one concerned with animal husbandry. If each of the blocks contains only $\frac{1}{2}, \frac{1}{4}$, etc., of the treatment combinations, some of the comparisons between the treatments are necessarily confounded with block differences in each replication, but, by confounding different comparisons in the different replications, some information on all comparisons may be obtained. It is the aim of the present paper to enumerate these possibilities of confounding, and, with this object in view, it is useful to consider first of all the structure of the systems.

§ 2. *The Structure of the 2^n System.*

A set of 2^n values of the treatment combinations can be replaced by any set of 2^n independent linear functions of these values. Such a set of linear functions is said to be an orthogonal one, if, for every pair of functions in the set, the sum of the products of the coefficients

of the corresponding values is zero. The mean, the main effects and the interactions form such a group.*

The structure can best be seen by setting out such a diagrammatic scheme as that given in Fig. 1. The 2^4 system has been used as an example, but precisely similar schemes will apply in all cases. In this arrangement there are sixteen different treatment combinations of the type a, b, c, d , etc. Each of the fifteen treatment comparisons denoted, as shown in the left-hand column of the table, by $A, B, C \dots$ is represented by one of the sets of signs given in the body of the table. The headings at the top of the table, indicating treatment combinations, are those appropriate to the assignment of the sets of signs. The symbols on the right-hand side and at the foot of the table will be referred to subsequently.

FIGURE 1.
The 2^4 system.

Treatment Comparisons (standard arrangement).	Treatment Combinations.																Treatment Comparisons (arbitrarily assigned)
	a_2								a_1								
	b_2				b_1				b_2				b_1				
	c_2		c_1		c_2		c_1		c_2		c_1		c_2		c_1		
	d_2	d_1	d_2	d_1	d_2	d_1	d_2	d_1	d_2	d_1	d_2	d_1	d_2	d_1	d_2	d_1	
A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	PQ
B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	RS
C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P
D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S
AB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	PQRS
AC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Q
AD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	PQS
BC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	PRS
BD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	R
CD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	PS
ABC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	QRS
ABD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	PQR
ACD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	QS
BCD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	PR
ABCD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	QR
	p_2	p_1	q_2	q_1	r_2	r_1	s_2	s_1	p_2	p_1	q_2	q_1	r_2	r_1	s_2	s_1	

* The main effect of any factor a has been defined as the mean of the responses to the factor a in the presence of all combinations of the remaining factors; i.e. with three factors a, b and c , the main effect of a is represented symbolically by the expansion of $\frac{1}{4}(a_2 - a_1)(b_2 + b_1)(c_2 + c_1)$.

The first-order interaction of a and b is similarly defined as one-half the mean of the differences in the responses to the factor a at the two levels of b in the presence of all combinations of the remaining factors; i.e. with the same three factors, the first-order interaction of a and b is represented by $\frac{1}{2}(a_2 - a_1)(b_2 - b_1)(c_2 + c_1)$. Similarly with higher-order interactions.

It will be seen that the interaction between any two main effects is represented by the set of signs obtained by multiplying together the corresponding signs of the pair of main effects considered, and that the same is true of any two treatment comparisons which have no treatment factor in common. This rule can be extended to include the generalized interaction of any two lines, provided that if the comparisons represented by these two lines have a factor, or factors, in common (such as AB and BC), these factors do not appear in the resultant generalized interaction (AC , in the example chosen).

This property implies that there is an internal symmetry in the table, such that if any appropriately chosen set of four (or, in the case of n treatment factors, n) lines be styled the main effects—the designation of the columns indicating treatment combinations being determined by the lines chosen—the remainder of the table will represent the interactions. The lines which can be “appropriately chosen” can be determined by successive steps. Thus if we now represent the treatment factors by p, q, r, s , and the treatment comparisons by the corresponding capital letters, any two lines may be taken to represent the main effects P and Q , respectively. The line assignable to the interaction PQ is then determined. Any one of the remaining lines may be chosen to represent R , the lines representing PR, QR , and PQR being then fixed. Finally any one of the remaining lines may be taken to represent S : the rest of the interactions are determined in accordance with this final selection. This process is indicated by the symbols on the right-hand side and at the foot of the table.

The initial choice need not be confined to main effects. The first selection might have been the assignment of the first two lines of the table to PQ and RS with the resultant determination of the fifth line as their interaction $PQRS$. Thence the same procedure as before might have been followed.

§ 3. *Confounding in the 2^n Systems.*

We may consider now the possibilities of confounding treatment comparisons when a 2^n system is arranged in $2, 2^2 \dots$ or 2^k blocks in each replication. A table similar to that already given for the 2^4 system may be supposed set out. If there are to be two blocks in each replication and, in any one replication, the treatment combinations represented by the first 2^{n-1} columns of the table are placed in the first block, while those represented by the second 2^{n-1} columns are placed in the second, then the treatment comparison represented by the first line of the table, which is the difference between the

total responses to treatment in the two blocks, is confounded with block differences in this replication.

If each of these blocks is divided into two halves, so that there are 2^2 blocks in each replication, and in any one replication the treatment combinations represented by the first 2^{n-2} columns are placed in the first block, etc., then the treatment comparisons represented by the first two lines and by the interaction between them, which represent the three possible comparisons which can be made between the blocks of this replication, are confounded. It is clear that the first two treatment comparisons may be chosen arbitrarily, and the third is then fixed, being their interaction.

In general, if there are to be 2^k blocks in each replication and in any one replication the treatment combinations represented by the first 2^{n-k} columns are placed in the first block, etc., then the comparisons represented by the first k lines and by the interactions between them are confounded in this replication. Thus when the main effects or interactions corresponding to the first k lines have been chosen, the remaining confounded interactions or main effects are uniquely determined. The selection of the first k comparisons is to be in accordance with the restrictions given above.

In any chosen design of the $2 \times 2 \times 2 \dots$ type, the possibilities of confounding can, therefore, be written down without difficulty. It is not always advantageous to select arbitrarily the highest-order interactions for confounding, since the generalized interactions of these among themselves may be first-order interactions, or even main effects, and these will then be confounded also. The choice depends on the type of experiment and the specific points which the experimenter is desirous of investigating, but, for the agriculturist at least, it is, in general, desirable to leave the main effects and as many as possible of the first-order interactions clear of block differences.

§ 4. *Possible Sets of Confounded Interactions.*

Table I shows for some of the simpler designs those typical sets of treatment comparisons which, in general, it will be best to confound in any one replication. These have been selected from all the sets possible, on the grounds that they involve no confounding of main effects and as little as possible of first-order interactions. The treatment factors and comparisons are denoted by the same series of symbols as in the earlier part of the paper. No arrangements involving the division of a replication into two blocks only have been included, since, in such divisions, it is possible to confound any one desired treatment comparison.

TABLE I.

Typical sets of treatment comparisons which may advantageously be confounded in some of the simpler of the $2 \times 2 \times 2 \dots$ designs.

Design.	Sets of Treatment Comparisons.
2^4 in 4 blocks of 4 treatments	$\left. \begin{array}{l} AB \\ CD \\ ABCD \end{array} \right\} \left. \begin{array}{l} AB \\ ACD \\ BCD \end{array} \right\}$
2^5 in 4 blocks of 8 treatments	$\left. \begin{array}{l} AB \\ CDE \\ ABCDE \end{array} \right\} \left. \begin{array}{l} ABC \\ ADE \\ BCDE \end{array} \right\} \left. \begin{array}{l} AB \\ ACDE \\ BCDE \end{array} \right\} \left. \begin{array}{l} AB \\ ACD \\ BCD \end{array} \right\}$
2^5 in 8 blocks of 4 treatments	$\left. \begin{array}{l} AB \\ AC \\ BC \\ ADE \\ BDE \\ CDE \\ ABCDE \end{array} \right\} \left. \begin{array}{l} AB \\ CD \\ ACE \\ ADE \\ BCE \\ BDE \\ ABCD \end{array} \right\}$
2^6 in 4 blocks of 16 treatments	$\left. \begin{array}{l} ABC \\ ADE \\ BCDE \end{array} \right\} \left. \begin{array}{l} ABC \\ DEF \\ ABCDEF \end{array} \right\} \left. \begin{array}{l} ABC \\ ADEF \\ BCDEF \end{array} \right\} \left. \begin{array}{l} ABCD \\ AB EF \\ CDEF \end{array} \right\}$
2^6 in 8 blocks of 8 treatments	$\left. \begin{array}{l} AB \\ CD \\ ABCD \\ ACEF \\ ADEF \\ BCEF \\ BDEF \end{array} \right\} \left. \begin{array}{l} BD \\ ABC \\ ACD \\ BEF \\ DEF \\ ACEF \\ ABCDEF \end{array} \right\} \left. \begin{array}{l} BD \\ ABC \\ ACD \\ CEF \\ ABEF \\ ADEF \\ BCDEF \end{array} \right\} \left. \begin{array}{l} ACE \\ BDE \\ BCF \\ ADF \\ ABCD \\ AB EF \\ CDEF \end{array} \right\}$
2^6 in 16 blocks of 4 treatments	$\left. \begin{array}{l} AB \\ CD \\ EF \\ ACE \\ BCE \\ ACF \\ BCF \\ ADE \\ BDE \\ ADF \\ BDF \\ ABCD \\ AB EF \\ CDEF \\ ABCDEF \end{array} \right\}$

Each set is typical of a number of exactly analogous ones which can be written down immediately by substituting the other treatment factors involved for those which are given in the table. If the experiment is a replicated one, a different group may be confounded in each replication, so that all the information on a particular group is not lost. The different groups need not be members of the one analogous set, but it is usually more convenient if they are so chosen.

If, for example, the 2^5 design arranged in eight blocks of four treatments suits the experimenter's requirements, the use of five replications will give a symmetrical layout in which one of the five analogous groups composed of two first-order, four second-order, and one third-order interaction may be confounded in each replication. With this arrangement each of the first- and third-order interactions occurs once, and each of the second-order interactions twice, in the complete experiment, so that $1/5$ of the information on each of the first- and third-order interactions, and $2/5$ on each of the second-order interactions is lost.

If the 2^6 design is arranged in eight blocks of eight treatments, and it is decided to confound analogous sets of comparisons composed of four second-order and three third-order interactions, it might be hoped that the use of five replications would give a symmetrical design. This, however, does not prove to be the case. The best that can be done with five replications is that one of the twenty second-order interactions shall occur twice, while a second does not occur at all, the remaining eighteen occurring once and once only. On the other hand, if the system is arranged in sixteen blocks of four treatments, it is possible with five replications to obtain a symmetrical arrangement in which one of the analogous sets composed of three first-order, eight second-order, three third-order and one fifth-order interaction (the sets typified by that given in the last line of Table I) is confounded in each replication. In this case $1/5$ of the information is lost on each of the fifteen first-order interactions, $2/5$ on each of the twenty second-order ones, $1/5$ on each of the fifteen third-order ones, while the fifth-order interaction is completely confounded.

There is, of course, no necessity for the arrangement to be balanced in the above manner; indeed, five replications of a 2^6 design involve more material than an experimenter is likely to have at his disposal. In an agricultural field trial, for example, 320 plots would be required, which implies a layout of greater magnitude than would commonly be feasible. This, however, does not detract from the interest of the more complicated designs. One of their chief uses is in making exploratory surveys of new material where the primary object is to ascertain which of a large number of possibly relevant factors exert effects of any kind, the interactions, with the possible exception of the first-order ones, being of secondary interest. In such cases even a single replication may provide valuable information, and if a small number of the first-order interactions are inevitably confounded, as in the case of the 2^6 arranged in blocks of four treatments, these may be allotted to these treatments whose effects so far as can be judged *a priori* are least likely to interact.

Some, or all, of the higher-order interactions can be used as an estimate of error.¹ The partition of degrees of freedom in the analyses of variance of a single replicate of the 2^6 system, arranged in blocks of eight treatments, and in blocks of four treatments, is given in Table II (*a* and *b*).

TABLE II.

Partition of degrees of freedom for a single replication of the 2^6 design arranged in eight blocks of eight treatments, and sixteen blocks of four treatments, respectively.

(*a*) Eight blocks of eight treatments.

(*b*) Sixteen blocks of four treatments.

Variance due to	Degrees of Freedom.	Variance due to	Degrees of Freedom.
Blocks	7	Blocks	15
Main effects	6	Main effects	6
First-order interactions	15	First-order interactions	12
Error	35	Error	30
Total	63	Total	63

When a given amount of material is available, it is frequently better to use such designs as these, in order that as many factors as possible may be included, rather than to use several replications of a design involving fewer factors, thus necessitating the omission of certain factors which may be relevant. L. H. C. Tippett has recently described similar arrangements, utilizing the properties of five by five hyper-graco-latin squares, which he used to investigate the effects of treatment factors, ignoring all interactions.² In all designs of this kind, preliminary investigation will show which of the factors suspected of being irrelevant may be ignored with confidence, and then, if the experimenter desires more information, replicated experiments can be performed with those retained.

§ 5. *Summary.*

The aim of the paper is to enumerate the confounded arrangements possible in a factorial design of the 2^n type, and to show how these may be obtained.

In the introductory section a brief account is given of some of the practical applications of these designs.

The second section deals with the structure of the 2^n system, and shows that the main effects and interactions are algebraically interchangeable, so that any set of signs in the schematic diagram given may be taken to represent any chosen set of treatment comparisons, provided that certain restrictions, made necessary by the

relation existing between any pair of comparisons and the generalized interaction between them, are not violated.

This leads to the general result given in the third section—namely, that if any one replication of a 2^n system is divided into 2^k equal blocks, then k treatment comparisons can be arbitrarily selected for confounding, the choice being governed only by the above-mentioned restrictions, and the remaining confounded treatment comparisons are then fixed, being the generalized interactions between the original k comparisons.

In the fourth section a table is given which shows, for some of the simpler designs of the 2^n type, typical sets of treatment comparisons which may advantageously be confounded in any one replication, and certain practical applications of these designs are briefly considered.

My thanks are due to Mr. F. Yates (Chief Statistician, Rothamsted Experimental Station) for the assistance he has given me throughout in the preparation of this manuscript.

References.

- ¹ Fisher, R. A., *The Design of Experiments*. Edinburgh: Oliver and Boyd, 1935.
- ² Tippett, L. H. C., *Applications of Statistical Methods to the Control of Quality in Industrial Production*. Manchester Statistical Society, 1936. pp. 1-32.

THE EFFICIENCIES OF THE BINOMIAL SERIES TESTS OF SIGNIFICANCE
OF A MEAN AND OF A CORRELATION COEFFICIENT.

By W. G. COCHRAN, B.A.

1. *Introduction.*

IN a preliminary survey of a set of data, one sometimes wishes to decide rapidly, without any elaborate calculation, whether there is any indication of a correlation between two sets of figures, or whether a set of differences appears to indicate a real effect. In such a case a rough test of significance is wanted, by means of which the data can be separated quickly into those effects which require further examination by more sensitive methods and those which can at once be set aside as either proved beyond dispute as real, or showing no signs of being real.

A convenient and familiar test of this type is provided by the binomial series distribution. In testing a set of differences, the numbers of positive and negative differences are counted, the sizes of the differences being ignored. If there is no real difference, and the errors are symmetrically distributed, positive and negative values should occur with equal frequency. The probability of obtaining r differences of the same sign out of n is

$${}^nC_r \frac{1}{2^n}$$

and the exact probability of obtaining r or more than r differences of the same sign is easily calculated. Table I shows, for values of

TABLE I.

Minimum Number (r) of Like Signs out of n Required to Reach 5 per cent. Significance.

<i>n.</i>	<i>r.</i>	<i>n.</i>	<i>r.</i>	<i>n.</i>	<i>r.</i>	<i>n.</i>	<i>r.</i>	<i>n.</i>	<i>r.</i>
—	—	11	10	21	16	31	22	41	28
—	—	12	10	22	17	32	22	42	28
—	—	13	11	23	17	33	23	43	29
—	—	14	12	24	18	34	24	44	29
—	—	15	12	25	18	35	24	45	30
6	6	16	13	26	19	36	25	46	30
7	7	17	13	27	20	37	25	47	31
8	8	18	14	28	20	38	26	48	32
9	8	19	15	29	21	39	26	49	32
10	9	20	15	30	21	40	27	50	33

Above $n = 50$, r may be taken as the smallest integer greater than $\left(\frac{n}{2} + \sqrt{n}\right)$.

n up to 50, the number of differences of like sign, either positive or negative, required to reach the 5 per cent. level of significance. These numbers do not, of course, correspond exactly to 5 per cent. probabilities, since the binomial distribution is discrete. The probability of obtaining 9 or more like signs out of 10 is, for example, 0.0215, but the probability of obtaining 8 or more is 0.109, which does not reach the 5 per cent. level of significance. The 5 per cent. level of significance is not reached until $n = 6$, even if all signs are the same.

The test may also be used in examining a possible correlation between two sets of figures. The method consists in finding the mean of each set, and noting the number of pairs of values whose deviations from the mean are of the same sign. If this is unusually large, a positive correlation is indicated; if it is unusually small, a negative one. This test is perhaps more useful than the corresponding test on differences, in that the exact calculation of the correlation coefficient is more laborious to make than the test of a mean difference.

An example of the use of the binomial series distribution in testing a set of differences has been given by Fisher,* and may also be used to illustrate the test of a correlation. Table II is taken from the data given by Fisher.

TABLE II.
*Additional Hours of Sleep Gained by the Use of
Hyoscyamine Hydrobromide.*

Patient.	1 (dextro-).	2 (lævo-).	Difference (2 - 1).
1	+ 0.7	+ 1.9	+ 1.2
2	- 1.6	+ 0.8	+ 2.4
3	- 0.2	+ 1.1	+ 1.3
4	- 1.2	+ 0.1	+ 1.3
5	- 0.1	- 0.1	0.0
6	+ 3.4	+ 4.4	+ 1.0
7	+ 3.7	+ 5.5	+ 1.8
8	+ 0.8	+ 1.6	+ 0.8
9	0.0	+ 4.6	+ 4.6
10	+ 2.0	+ 3.4	+ 1.4
Mean (\bar{x})	0.75	+ 2.33	+ 1.58

One of the differences is zero, but the remaining nine are all positive, which points strongly to a real superiority of Lævo- over Dextro-. The significance level indicated by this test is $2/2^9$ or 1 in 256, whereas the exact level given by the t test is about 1 in 700. We might also wish to test whether the patients' responses to the two types of drug were correlated. It is easily verified from Table II that the responses to the two drugs lie on the same sides of their

* R. A. Fisher, *Statistical Methods for Research Workers*, § 24. Edinburgh: Oliver and Boyd.

respective mean responses, except for patients 8 and 9. Thus there are 8 similar responses out of 10. This does not reach the 5 per cent. significance level, as reference to Table I shows, but it is sufficiently near to call for further examination by the exact test of significance. We find $r = 0.795$, which is significant at the 1 per cent. point.

The above is not a good illustration of the practical circumstances in which the tests would be useful. The number of pairs of observations (10) is small, and one would be reluctant to discard any information; further, for the same reason, the exact tests of significance can be made without labour. It is when the number of observations is large, say over 40, so that some information may be spared and the exact tests are more laborious, that the approximate tests will be useful in a preliminary analysis.

The object of this note is to compare the efficiencies of these tests with those of the exact tests, *i.e.* to determine how much information is thrown away by ignoring the sizes of the differences or of the deviations from the means. The point is of some importance, because if the tests prove to be of low efficiency, further examination may still be required when they give a result of non-significance.

2. *The Efficiency of the Binomial Series Test of a Difference.*

If the differences vary normally and independently about a mean μ , with standard deviation σ , the probability p of obtaining a positive difference is

$$\begin{aligned} p &= \frac{1}{\sigma\sqrt{2\pi}} \int_0^{\infty} e^{-\frac{(x-\mu)^2}{2\sigma^2}} dx \\ &= \frac{1}{\sqrt{2\pi}} \int_{-\frac{\mu}{\sigma}}^{\infty} e^{-y^2/2} dy \end{aligned}$$

Hence, by estimating p from the observed distribution of positive and negative signs, we obtain an estimate of $\frac{\mu}{\sigma} = \tau$ (say)

$$\text{Now,} \quad \delta p = + \frac{1}{\sqrt{2\pi}} e^{-\frac{\tau^2}{2}} \delta \tau$$

$$\text{Hence} \quad V(\tau) = 2\pi e^{\tau^2} V(p) = 2\pi e^{\tau^2} pq/n.$$

An efficient estimate of $\frac{\mu}{\sigma}$ is

$$\tau = \frac{\bar{x}}{\sqrt{\frac{S(x-\bar{x})^2}{n-1}}} = \frac{t}{\sqrt{n}}, \text{ where } t \text{ is Student's } t.$$

$$\text{And} \quad V(\tau) = \frac{1}{n-2}.$$

The efficiency of estimation by the binomial series is given by the ratio of the variances of $\hat{\tau}$ and τ when n is large.

$$\text{Efficiency} = \frac{V(\hat{\tau})}{V(\tau)} = \frac{1}{2\pi p q e^{1/2}}.$$

Table III shows the percentage efficiency of estimation for a series of values of $\frac{\mu}{\sigma}$.

TABLE III.

Percentage Efficiency of Estimation of $\frac{\mu}{\sigma}$ by Taking Account of Signs Only.

$\frac{\mu}{\sigma}$	0	± 1	± 1	$\pm 1\frac{1}{2}$	± 2	$\pm 2\frac{1}{2}$	± 3
	63.7	58.1	43.9	26.9	13.1	5.0	1.5

The efficiency is greatest when $\frac{\mu}{\sigma} = 0$ at which it has the value $\frac{2}{\pi}$. Thereafter it decreases steadily to zero. This is to be expected, since when $\frac{\mu}{\sigma}$ is large, the signs of the differences will all be positive except very rarely, and the estimate of $\frac{\mu}{\sigma}$ will almost always be $+\infty$.

The efficiency of the test as a test of significance is its efficiency of estimation when $\frac{\mu}{\sigma} = 0$, and is $\frac{2}{\pi}$, i.e. just under $\frac{1}{2}$. Thus about $\frac{1}{2}$ of the information is discarded by taking account of signs only.

3. The Efficiency of the Binomial Series Test of a Correlation Coefficient.

We may assume that the pairs of values, x and y , are distributed about zero in a bivariate normal distribution. The probability of obtaining two deviations of like sign is

$$p = \frac{1}{2\pi\sigma_1\sigma_2\sqrt{1-\rho^2}} \int_{-\infty}^0 \int_{-\infty}^0 + \int_0^{\infty} \int_0^{\infty} e^{-\frac{1}{2(1-\rho^2)} \left(\frac{x^2}{\sigma_1^2} - \frac{2\rho xy}{\sigma_1\sigma_2} + \frac{y^2}{\sigma_2^2} \right)} dx dy$$

This may be written

$$p = \frac{1}{\pi\sqrt{1-\rho^2}} \int_0^{\infty} \int_0^{\infty} e^{-\frac{1}{2(1-\rho^2)} (x^2 - 2\rho xy + y^2)} dx dy$$

so that the value of p gives an estimate of the correlation coefficient ρ .

$$\begin{aligned}
 p &= \frac{1}{\pi\sqrt{1-\rho^2}} \int_0^\infty \int_0^\infty e^{-\frac{1}{2}v^2} e^{-\frac{1}{2}\frac{(x-\rho y)^2}{1-\rho^2}} dx dy \\
 &= \frac{1}{\pi} \int_0^\infty e^{-\frac{1}{2}v^2} dy \int_{\frac{-\rho y}{\sqrt{1-\rho^2}}}^\infty e^{-\frac{1}{2}u^2} du \\
 &= \frac{1}{2} + \frac{1}{\pi} \int_0^\infty e^{-\frac{1}{2}v^2} dy \int_0^{\frac{\rho y}{\sqrt{1-\rho^2}}} e^{-\frac{1}{2}u^2} du.
 \end{aligned}$$

This may be written

$$\frac{1}{2} + \frac{1}{\pi} \iint e^{-\frac{1}{2}(x^2+v^2)} dx dy$$

over the sector of the x, y plane in which θ lies between 0 and $\sin^{-1} \rho$. A change to polar co-ordinates gives at once

$$p = \frac{1}{2} + \frac{\sin^{-1} \rho}{\pi}$$

Hence

$$\frac{dp}{d\rho} = \frac{1}{\pi\sqrt{1-\rho^2}}$$

Hence if ρ_b is the estimate of ρ

$$V(\rho_b) = \pi^2(1-\rho^2) V(p) = \frac{\pi^2(1-\rho^2)^2 pq}{n}$$

The variance of an efficient estimate of ρ is $\frac{(1-\rho^2)^2}{n-1}$, so that the efficiency of estimation of ρ by the binomial series distribution is

$$E = \frac{(1-\rho^2)}{\pi^2 pq}$$

The percentage efficiencies for a series of values of ρ are shown in Table IV.

TABLE IV.

Percentage Efficiency of Estimation of ρ by Taking Account of Signs Only.

ρ	0.0.	± 0.1 .	± 0.2 .	± 0.3 .	± 0.4 .	± 0.5 .	± 0.6 .	± 0.7 .	± 0.8 .	± 0.9 .	± 1.0 .
E	40.5	40.3	39.6	38.3	36.6	34.2	31.2	27.5	22.4	15.6	0.0

The efficiency decreases slowly from the value $\frac{4}{\pi^2}$, at $\rho = 0$, to zero at $\rho = \pm 1$. The efficiency of the binomial series test of significance is 40 per cent. This rather low figure indicates that the test must be used with caution in setting aside data on the grounds that there is no apparent correlation. A glance at the sizes of the deviations from the mean will, however, generally enable one to decide whether the question requires further investigation.

PROBLEMS ARISING IN THE ANALYSIS OF A SERIES OF SIMILAR EXPERIMENTS.

By W. G. COCHRAN, B.A.

§ 1. *Introduction.*

AN efficient type of modern field experiment is that in which a replicated trial is laid down in the same year at a number of centres, or carried out at the same centre independently throughout a number of years. The statistical problems which arise in the interpretation of the results of such a set of data are of wide generality. For any treatment effect, we obtain at each centre an estimate x and an estimate s of its standard error, based on n degrees of freedom. As a preliminary to more detailed examination, the experimenter wants to estimate and test the significance of the mean treatment effect and to find whether it has varied from centre to centre.

If the individual experiments are assumed to be equally accurate and the estimated standard errors do not contradict this assumption, the statistical treatment is easy and familiar. If there are k centres, the data for any particular treatment response may be analysed into

					D.F.
Mean response	1
Interaction of response with centres	($k-1$)	
Local experimental error	nk	

The interaction of the treatment response may be tested against the combined experimental errors, and the mean response may be tested against either the interaction or the experimental error. The interpretation of these two tests has been discussed by Fisher (1).

It will, however, be the exception to find the individual experiments all of the same precision. The object of this paper is to discuss the estimation of the mean response and the test of significance of the interaction of the response with centres, where we do not wish to assume that the standard errors are all equal.

§ 2. *The Equations of Estimation.*

The most general hypothesis to be considered is that the treatment response at any centre is the sum of two parts, each normally and independently distributed; one, representing the contribution of local experimental errors, varies about zero with standard deviation σ_1 , while the other, which represents the responsiveness of the centre to the treatment, varies about a general mean μ with standard deviation σ . The parameters μ and σ are the same for all centres, but

σ_i varies from centre to centre. An estimate s_i of σ_i , based on n degrees of freedom, is available from the local analysis of variance.

For a single centre, the joint sampling distribution of s_i and x_i may be written, apart from the constant of integration

$$\frac{s_i^{n-1}}{\sigma_i^n \sqrt{\sigma^2 + \sigma_i^2}} e^{-\frac{1}{2} \left\{ \frac{ns_i^2}{\sigma_i^2} + \frac{(x_i - \mu)^2}{\sigma^2 + \sigma_i^2} \right\}} dx_i ds_i$$

Hence the logarithm of the likelihood for all centres is

$$L = -nS \log \sigma - \frac{1}{2} S \log (\sigma^2 + \sigma_i^2) - \frac{1}{2} S \left\{ \frac{ns_i^2}{\sigma_i^2} + \frac{(x_i - \mu)^2}{\sigma^2 + \sigma_i^2} \right\}$$

the sum being taken over all k centres.

The equations of estimation of μ , σ_i , σ are

$$\frac{\partial L}{\partial \mu} = S \frac{(x_i - \mu)}{\sigma^2 + \sigma_i^2} = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$\frac{\partial L}{\partial \sigma_i} = -\frac{n}{\sigma_i} - \frac{\sigma_i}{\sigma^2 + \sigma_i^2} + \frac{ns_i^2}{\sigma_i^3} + \frac{\sigma_i(x_i - \mu)^2}{(\sigma^2 + \sigma_i^2)^2} = 0 \quad . \quad (2)$$

$$\frac{\partial L}{\partial \sigma} = -S \frac{\sigma}{\sigma^2 + \sigma_i^2} + S \frac{\sigma(x_i - \mu)^2}{(\sigma^2 + \sigma_i^2)^2} = 0 \quad . \quad . \quad . \quad (3)$$

The equations are complicated and have no simple general solution. The complication is mainly due to the fact that the value of x_i provides information about all three parameters μ , σ , and σ_i .

§ 3. The Estimation of the Response when it is Assumed Constant at all Centres.

If we assume $\sigma = 0$, the equations of estimation of μ and σ_i give

$$S \frac{(x_i - \mu)}{\sigma_i^2} = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

$$\sigma_i^2 = \frac{ns_i^2 + (x_i - \mu)^2}{n + 1} \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

Thus the equation of estimation of the mean response is

$$S \frac{x_i - \mu}{s_i^2 + \frac{(x_i - \mu)^2}{n}} = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

If the values of σ_i were known exactly, the sufficient estimate of the mean would be given by the solution of

$$S \frac{(x_i - \mu)}{\sigma_i^2} = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

i.e. by the weighted mean. Where the σ_i have also to be estimated, we do not simply replace σ_i by s_i in equation (7) to obtain an efficient estimate, but make use of the extra information about σ_i contained in

x_i . Equation (6) may be solved fairly quickly by successive approximation, starting with the unweighted mean as a first estimate.

To find the amount of information in the estimated mean and perform tests of significance, it is necessary to calculate the sampling variance of the solution, $\hat{\mu}$ say, of equation (6). For a given set of σ_i and μ , this would at first sight appear to depend on all these unknown parameters, but Bartlett (2) has shown how to use the information available about the unknown σ_i to obtain the sampling variance of $\hat{\mu}$ in terms of the single unknown μ . In the joint distribution of x and s at any centre

$$C s^{n-1} e^{-\frac{1}{2\sigma^2} \{ns^2 + (x-\mu)^2\}} dx ds \quad . \quad . \quad . \quad (8)$$

he writes $\Sigma = ns^2 + (x - \mu)^2$ and substitutes for s in terms of Σ . This gives for the joint distribution of x and Σ

$$C \Sigma^{\frac{n}{2}-1} \{1 - (x - \mu)^2/\Sigma\}^{\left(\frac{n}{2}-1\right)} e^{-\frac{x}{\sigma_i}} dx d\Sigma \quad . \quad . \quad (9)$$

It will be noted that the distribution of x for fixed Σ depends only on the unknown mean μ . Thus the variance of $\hat{\mu}$ for a fixed Σ , which is known if μ is known, will depend only on the unknown mean μ , and not on the σ_i .

To find the variance of $\hat{\mu}$, write

$$X(\hat{\mu}) = S \frac{(x - \hat{\mu})}{ns^2 + (x - \hat{\mu})^2} = 0 \quad . \quad . \quad . \quad (10)$$

Now

$$0 = X(\hat{\mu}) = X(\mu) + (\hat{\mu} - \mu) \frac{\partial X}{\partial \mu} + \quad . \quad . \quad . \quad (11)$$

Thus if the number of centres is large,

$$E(X^2) = \sigma_\mu^2 E^2 \left(\frac{\partial X}{\partial \mu} \right) \quad . \quad . \quad . \quad . \quad (12)$$

From equation (9), the variance of x for fixed Σ is $\Sigma/n + 1$. Thus

$$E(X^2) = S \left\{ E \left(\frac{(x - \mu)^2}{\Sigma^2} \right) \right\} = \frac{1}{(n+1)} S \left(\frac{1}{\Sigma} \right) \quad . \quad (13)$$

and

$$E \left(\frac{\partial X}{\partial \mu} \right) = S \left\{ E \left(\frac{(x - \mu)^2}{\Sigma^2} - \frac{1}{\Sigma} \right) \right\} = - \left(\frac{n-1}{n+1} \right) S \left(\frac{1}{\Sigma} \right) \quad (14)$$

so that

$$\sigma_\mu^2 = \frac{(n+1)}{(n-1)^2} 1/S \left(\frac{1}{\Sigma} \right) \quad . \quad . \quad . \quad (15)$$

The average amount of information for a fixed set of σ_i is $E \left(\frac{1}{\sigma_\mu^2} \right)$ and is easily found to be

$$\left(\frac{n-1}{n+1} \right) S \left(\frac{1}{\sigma^2} \right) \quad . \quad . \quad . \quad . \quad (16)$$

Thus the average fraction of information lost through the inaccuracy of the weights is $\frac{2}{n+1}$.

An alternative which suggests itself to the use of the maximum likelihood solution is the weighted mean

$$\mu_w = S\left(\frac{x}{s^2}\right) / S\left(\frac{1}{s^2}\right) \quad . \quad . \quad . \quad . \quad (17)$$

This has the advantage that it can be calculated directly and is a familiar type of mean in statistical work, and it is worth while estimating its efficiency. For a fixed set of s_i ,

$$V(\mu_w) = S\left(\frac{\sigma^2}{s^4}\right) / S^2\left(\frac{1}{s^2}\right) \quad . \quad . \quad . \quad . \quad (18)$$

so that a knowledge of the s_i does not, in this case, enable us to dispense with the knowledge of the σ_i , though it has the advantage that for fixed s_i , μ_w is normally distributed. Since

$$E\left(\frac{1}{s^2}\right) = \frac{n}{n-2} \frac{1}{\sigma^2} \text{ and } E\left(\frac{1}{s^4}\right) = \frac{n^2}{(n-2)(n-4)} \frac{1}{\sigma^4}$$

the average variance of μ_w for a fixed set of σ_i is, provided $n > 4$,

$$\left(\frac{n-2}{n-4}\right) 1/S\left(\frac{1}{\sigma^2}\right) \quad . \quad . \quad . \quad . \quad (19)$$

Thus the amount of information in the weighted mean is

$$\left(\frac{n-4}{n-2}\right) S\left(\frac{1}{\sigma^2}\right) \quad . \quad . \quad . \quad . \quad (20)$$

Comparison with (16) shows that the superior efficiency of the maximum likelihood solution is equivalent to having 3 extra degrees of freedom in the estimates s_i .

For $n = 4$, expression (19) gives an infinite value for the variance of the weighted mean μ_w . This is not correct, since (18) shows that for a fixed set of σ_i the variance of μ_w cannot exceed the greatest of the variances σ_i^2 , i.e. the weighted mean cannot do worse than give as the estimate of μ the most variable single value x_i . For $n = 4$, the average variance of μ_w can be shown to be

$$S\left(\frac{1}{s^2}\right) / S\left(\frac{1}{s^2}\right) \quad . \quad . \quad . \quad . \quad (21)$$

This value lies between the most and least accurate of the individual estimates at the various centres. What is happening is that individual low values of s_i are turning up so frequently that usually all the information about μ is being derived from a single centre. Thus the percentage information retained tends to zero as the number of centres increases.

The percentage efficiencies of $\hat{\mu}$ and μ are shown for small values of μ in the table below.

TABLE I.
Percentage Efficiencies of $\hat{\mu}$ and μ_w .

n	2	3	4	5	6	8	10	15	20
$\hat{\mu}$	33	50	60	67	71	78	82	88	90
μ_w	0	0	0	33	50	67	75	85	89

For n greater than 15 there is little to choose between the two estimates, but for n less than 10 the increase in efficiency of the maximum likelihood solution is worth the extra labour. For values of n between 2 and 6 a good deal of information is being lost in the process of estimation of the weights. As these cases may be of practical importance (*e.g.* $n = 2$ might represent a set of 3×3 Latin squares), it is worth considering the relative efficiency of two other types of mean which suggest themselves.

One is the unweighted mean. This always retains a finite fraction of the information, the fraction decreasing as the true accuracies of the individual experiments diverge, and is not subject to any loss due to estimation of weights. The other method is to fix arbitrarily an upper limit to the weights, and below that to weight inversely as the estimated variance. This is equivalent to recognizing that in practice there is a limit to the accuracy with which an individual experiment may be carried out, and that very low values of s_i are likely to be under-estimates of the corresponding σ_i . The method has the advantage that no single experiment exerts too predominating an influence on the mean, while bad experiments are properly scaled down in weight; on the other hand, it has an element of arbitrariness in the choice of the upper limit. A comparison of the four types of mean for low values of n is given in the next section, the number of centres being assumed to be large.

§ 4. *The Relative Efficiencies of Four Types of Mean.*

The relative efficiencies of the weighted mean and the maximum likelihood solution have been shown to be $\left(\frac{n-4}{n-2}\right)$ and $\left(\frac{n-1}{n+1}\right)$ respectively, where n is the number of degrees of freedom in the estimates s_i . The variance of the unweighted mean is

$$\frac{1}{k^2} S \sigma_i^2$$

where k is the number of centres, and can be calculated for any given set of σ_i .

To calculate the efficiency of the weighted mean with an arbitrary upper limit, let the minimum true error variance be guessed as σ_0^2 . Then in sampling from a set of experiments in which the true variance is σ^2 , we take the weight w as $\frac{1}{\sigma_0^2}$ whenever $s^2 \leq \sigma_0^2$ and as $\frac{1}{s^2}$ whenever $s^2 > \sigma_0^2$. The variance of the mean $S(wx)/S(w)$ for any given set of σ_i^2 and fixed weights is

$$S(w^2\sigma^2)/S^2(w)$$

and the average variance for a given set of σ_i^2 is

$$S(\bar{w}^2\sigma^2)/S^2(\bar{w})$$

For $n = 6$, for example, the probability that $s^2 \leq \sigma_0^2$ is, (cf. (3)),

$$P(s^2 \leq \sigma_0^2) = 1 - e^{-\frac{1}{2}\chi^2} \left\{ 1 + \frac{1}{2}\chi^2 + \frac{1}{2!} \left(\frac{1}{2}\chi^2 \right)^2 \right\}$$

where $\frac{1}{2}\chi^2 = 3\sigma_0^2/\sigma_i^2$.

We require also the mean value of $\frac{1}{s^2}$ in the range (σ_0, ∞) . This is found to be

$$\frac{3}{2\sigma_0^2} e^{-\frac{1}{2}\chi^2} \{ 1 + \frac{1}{2}\chi^2 \}$$

Thus

$$w = \left[1 - e^{-\frac{1}{2}\chi^2} \left\{ 1 + \frac{1}{2}\chi^2 + \frac{1}{2!} \left(\frac{1}{2}\chi^2 \right)^2 \right\} \right] \sigma_0^2 + \frac{3}{2\sigma_0^2} e^{-\frac{1}{2}\chi^2} \left\{ 1 + \frac{1}{2}\chi^2 \right\}$$

Similarly

$$w^2 = \left[1 - e^{-\frac{1}{2}\chi^2} \left\{ 1 + \frac{1}{2}\chi^2 + \frac{1}{2!} \left(\frac{1}{2}\chi^2 \right)^2 \right\} \right] \left| \sigma_0^4 + \frac{9}{2\sigma_0^4} e^{-\frac{1}{2}\chi^2} \right|$$

The relative efficiencies of these four types of mean will depend on the distribution of experimental errors σ_i^2 . To obtain some actual figures, the efficiencies have been calculated for some sets of hypothetical values of σ_i^2 which are intended to cover the range likely to occur in practice. It is assumed that the k centres are divided into three groups as regards accuracy: a number λk have the same experimental variance lv , μk have variance mv , while the remaining νk have variance nv . We have

$$\lambda + \mu + \nu = 1 \text{ and } l < m < n$$

The sets of values assigned to (λ, μ, ν) are: (0.2, 0.6, 0.2) and (0.3, 0.4, 0.3), the first set representing, for instance, the case in which 60 per cent. of the experiments have the same accuracy, while 20 per cent. are less accurate and 20 per cent. more accurate. For each of these sets, (l, m, n) have been given the values $(\frac{1}{2}, 1, 2)$ and $(\frac{1}{3}, 1, 4)$ respectively. The case $(\lambda, \mu, \nu) = (0.2, 0.6, 0.2)$, $(l, m, n) = (\frac{1}{2}, 1, 2)$ means, for instance, that $\frac{1}{2}$ of the centres have experimental

variances $\frac{1}{2}v$, $\frac{2}{3}v$ have variances v and the remaining $\frac{1}{3}$ have variances $2v$. The four cases resulting cover a fairly wide range of variation in the accuracy of individual experiments, the relative accuracy of the best and the worst experiments ranging from 4 to 16.

Comparison of the four types of mean suggested is made in Table II for $n = 6, 4$ and 2 .

TABLE II.
Relative Efficiencies of Four Types of Mean.

n .	Relative Accuracy of the Groups (l, m, n).	Proportion in Groups (λ, μ, ν).	Maximum Likelihood Solution.	Weighted Mean.	Weighted Mean with Upper Limit.				Un-weighted Mean.
					$\frac{2}{v}$.	$\frac{1}{v}$.	$\frac{2}{3v}$.	$\frac{1}{2v}$.	
6	$\frac{1}{2}, 1, 2$	0.2, 0.6, 0.2 0.3, 0.4, 0.3	71	50	64	82	85	86	83
			71	50	62	81	82	82	76
4	$\frac{1}{2}, 1, 2$	0.2, 0.6, 0.2 0.3, 0.4, 0.3	60	0	67	78	83	83	83
			60	0	68	76	80	79	76
2	$\frac{1}{2}, 1, 2$	0.2, 0.6, 0.2 0.3, 0.4, 0.3	33	0	60	74	78	82	83
			33	0	60	70	74	77	76
6	$\frac{1}{4}, 1, 4$	0.2, 0.6, 0.2 0.3, 0.4, 0.3	71	50	69	72	71	69	48
			71	50	73	75	71	67	36
4	$\frac{1}{4}, 1, 4$	0.2, 0.6, 0.2 0.3, 0.4, 0.3	60	0	60	63	65	65	48
			60	0	63	63	62	60	36
2	$\frac{1}{4}, 1, 4$	0.2, 0.6, 0.2 0.3, 0.4, 0.3	33	0	44	49	53	56	48
			33	0	42	45	47	48	36

As the upper limit to the possible weights decreases from infinity (no adjustment) to zero, the efficiency increases from the value given by the weighted mean to a maximum, and thereafter decreases to the value given by the unweighted mean. Owing to the variability of the estimated weights, the maximum efficiency does not coincide with the case in which the upper limit is guessed correctly, but with a somewhat lower value. The efficiency is not very sensitive to variation in the point at which the upper limit is fixed, provided that it is not fixed too high.

Where $(l, m, n) = (\frac{1}{2}, 1, 2)$, so that the experiments do not vary widely in accuracy, there is little to choose between the unweighted mean and the weighted mean with fixed upper limit, the maximum likelihood solution being definitely inferior. With the wider variations in precision, the weighted mean with fixed upper limit is the most accurate, and for $n = 4$ or 6 the maximum likelihood solution comes next.

To sum up the question of estimation of a mean response where

it is assumed that there is no variation in response from experiment to experiment, the weighted mean, weighting inversely as the estimated variances, may be recommended if 15 or more degrees of freedom are available in the estimates of the weights. With less than 15 degrees of freedom one of the other means is advisable, and each has something in its favour.

The maximum likelihood solution is satisfactory from the point of view of information for values of n as low as 6, though it is slightly more tedious to calculate than the other means. The unweighted mean has simplicity to commend it, and is particularly suitable with sets of experiments which do not vary widely in precision and with low values of n . For values of n below 6 the weighted mean with a fixed upper limit is the most accurate of the three, if the limit can be chosen to represent the accuracy of the best group of experiments. The difficulty of assigning a standard error to this mean, is, however, a serious disadvantage.

§ 5. *The Test of Significance of the Mean Response.*

The tests given here are strictly appropriate to a large number of centres; the mean is assumed to be normally distributed, and is compared in the normal probability table with an unbiased estimate of its variance. In the analogous case of equal precision, the approximation is equivalent to replacing the t -distribution for nk degrees of freedom by the normal distribution. The agreement between the exact and approximate tests may not be as good with unequal as with equal variances, but may be expected to be satisfactory unless k is small. The case $k = 2$ is being investigated.

The estimated variances of the weighted mean and the maximum likelihood solution have already been found. The average variance of the weighted mean is $\binom{n-2}{n-4} S\left(\frac{1}{\sigma^2}\right)$. Replacing $S\left(\frac{1}{\sigma^2}\right)$ by an unbiased estimate in terms of s^2 , we get for the standard error

$$\sqrt{\frac{n}{n-4}} \sqrt{S\left(\frac{1}{s^2}\right)}$$

In the maximum likelihood solution, the estimate of the experimental variance σ_e^2 at any centre is taken as

$$\{ns_i^2 + (x_i - \bar{\mu})^2\}/(n + 1)$$

and the standard error of the mean may be written

$$\binom{n+1}{n-1} \sqrt{S\left(\frac{n+1}{ns^2 + (x - \bar{\mu})^2}\right)}$$

where $\bar{\mu}$ is the estimate of the mean.

The estimated standard error of the unweighted mean \bar{x} is

$$s_{\bar{x}} = \sqrt{S(s^2)/k}$$

It should be noted that the distribution of $\frac{\bar{x}}{s_{\bar{x}}}$ depends on the ratios of the unknown σ^2 , and is not that of Student's t unless the σ^2 are all equal. Where the product nk is sufficiently small that a t -test is indicated, a first approximation to the exact test may be found by a device which has been used by Fairfield Smith (4). The variance of any individual estimate s^2 is

$$V(s^2) = 2\sigma^4/n$$

Hence an estimate of the variance of s_x^2 is

$$V(s_x^2) = \frac{2}{nk^2} S(s^4)$$

But if n_x is the number of degrees of freedom appropriate to s_x , an estimate of $V(s_x^2)$ is

$$\frac{2}{n_x} s_x^4 = 2S^2(s^2)/n_x k^2$$

Thus the relative precision of $s_{\bar{x}}$ is estimated by assigning to it a number of degrees of freedom equal to

$$n_{\bar{x}} = nS^2(s^2)/S(s^4)$$

This number only attains its maximum, nk , if all experiments have the same s^2 . It reaches its minimum, n , if a single centre is much less accurate than any of the others, and in this case indicates, quite correctly, a t -test against n instead of nk degrees of freedom. In general, the integral part of $n_{\bar{x}}$ may be taken as the number of degrees of freedom in $s_{\bar{x}}$. No examination of the closeness of this approximation has yet appeared in print, but consideration of the case with two centres only indicates that the approximation may over-estimate on the average the probability of a deviation arising by chance.

I have been unable to find any method of obtaining a simple estimate of the standard error of the weighted mean with fixed upper limit. Even if a method were found, a weighted mean with a badly chosen upper limit would be assigned an under-estimate of its standard error. Unless this difficulty can be satisfactorily overcome, this mean is ruled out where an exact test of significance is required.

§ 6. *The Test of Significance of the Variation in Response from Centre to Centre.*

A test could be obtained by solving equations (1), (2) and (3) for σ and using the solution, $\hat{\sigma}$, as a test criterion, the significance levels

being obtained from the frequency distribution of δ when $\sigma = 0$. The test would be efficient if the mathematical specification of the problem set up in § 2 conformed to practice, but in any case the method cannot be used owing to the complexity of the equations.

If the mean response μ were known, the values $(x - \mu)/s$ would, in the absence of any variation in response, be distributed as t with n degrees of freedom, and a sensitive test could presumably be based on the value of

$$Q = S \frac{1}{s^2} (x - \mu)^2$$

Where the value of μ is unknown, analogy with the analysis of variance suggests that the appropriate estimate of it for this purpose is the weighted mean $\mu_w = S \frac{x}{s^2} / S \frac{1}{s^2}$, which is the value of μ which minimizes Q . With this value inserted, Q may be written

$$Q = S \frac{(x - \mu)^2}{s^2} - (\mu_w - \mu)^2 S \frac{1}{s^2}$$

The efficiency of this quantity as a test criterion will depend on the type of variation in response which occurs in practical applications, but it seems reasonable on common-sense grounds. It may be noted that the same type of expression is used to test the departure from independence in a $2 \times N$ contingency table (cf. (5), § 21).

If the weights were known exactly, Q would follow the χ^2 distribution with $(k - 1)$ degrees of freedom, k being the number of centres. In general, Q may be written

$$Q = S(t^2) - S^2\left(\frac{t}{s}\right) / S\left(\frac{1}{s^2}\right)$$

so that it is distributed as the sum of the squares of k values of t , less a correction term which is a weighted mean of the values of t . A good approximation to the distribution of Q should be obtained by replacing the weighted mean in the correction term by an unweighted mean. In particular

$$\begin{aligned} \text{Mean } (Q) &= \left(\frac{n}{n-2}\right) \left\{k-1 - \frac{4}{n(n-4)}\right\} \\ &\approx \text{Mean } \{S(t - \bar{t})^2\} - \left(\frac{n}{n-2}\right) (k-1) \end{aligned}$$

so that in replacing Q by $S(t - \bar{t})^2$ we are probably tending to over-estimate slightly the probability of a discrepancy arising by chance.

Further

$$V\{S(t - \bar{t})^2\} = 2 \left(\frac{n}{n-2}\right)^2 (k-1) \left(\frac{n-1}{n-4}\right) \left\{1 - \frac{3}{k(n-1)}\right\}$$

Thus $\left(\frac{n-2}{n}\right)S(t-i)^2$ has the same mean as χ^2 with $(k-1)$ degrees of freedom, but its variance is too large, approximately in the ratio $\left(\frac{n-1}{n-4}\right)$. A transformation which leads to the same mean and variance as χ^2 is obtained by putting

$$\chi_{\pi}^2 = (k-1) + \sqrt{\frac{n-4}{n-1}} \left\{ \left(\frac{n-2}{n}\right)S(t-i)^2 - (k-1) \right\}$$

The distribution of χ_{π}^2 and the tabular χ^2 tend to the same normal distribution, for any value of n , as k tends to infinity; they also tend to coincidence, for any k , as n tends to infinity, and have the same mean and variance for all values of n and k , except for very small values of both, in which the additional factor $\left(1 - \frac{3}{k(n-1)}\right)$ may be brought into the transformation. The agreement between the distribution of χ_{π}^2 and χ^2 may be expected to be at its worst for low values of both, since χ_{π}^2 has a lower limit $(k-1)\left(1 - \sqrt{\frac{n-4}{n-1}}\right)$ instead of zero. The difference even here is likely to be small for moderate values of n and k ; for $n=10$, $k=11$, for instance, the lower limit of χ_{π}^2 is 1.835 and the probability of getting a value of χ^2 lower than this for 10 degrees of freedom is only 0.0025.

It is therefore suggested that the transformation

$$\chi^2 = (k-1) + \sqrt{\frac{n-4}{n-1}} \left\{ \left(\frac{n-2}{n}\right)Q - (k-1) \right\}$$

may be used in testing the significance of the variation in response from centre to centre.

For a given value of n , this approximation will be worst when there are only two centres. The difference is, however, still on the side of declaring too few significant results, for $Q = \frac{(x_1 - x_2)^2}{s_1^2 + s_2^2}$ and the Q, χ^2 transformation is based on the t^2 distribution with n degrees of freedom, whereas the exact distribution of Q is probably better approximated by using in t^2 a number of degrees of freedom lying between n and $2n$, as indicated by the method suggested in § 5. Even with only two centres the Q, χ^2 transformation will thus avoid the danger of obtaining too many significant results, though the method of determining the equivalent number of degrees of freedom is to be recommended as more sensitive, and further work on this important particular case is needed.

In general, the use of Q as a test criterion is inadvisable for values of n below 6; the Q, χ^2 transformation breaks down when $n=4$, and the variance of the t -distribution itself is infinite when $n=2$.

In these cases it is best to obtain the individual values of $t = (x - \mu)/s$ and compare these with the tabulated t -distribution. The estimate of μ used should be one of the three other types of mean suggested, but not the weighted mean. The transformation from Q to χ^2 cannot, however, be used with these means, since they will always give higher values of Q than the weighted mean.

§ 7. *Estimation of the Mean Response when it Varies from Centre to Centre.*

If it cannot be assumed that the interactions do not exist, the question of estimation of the mean response is more difficult. Equation (1)

$$S \frac{(x_i - \mu)}{\sigma^2 + s_i^2} = 0$$

indicates that a kind of semi-weighted mean is appropriate, but the complete solution of equations (1), (2) and (3) would be very tedious. If a fairly efficient solution is required in a particular case, there will probably be very little information lost if the s_i^2 are used as estimates of σ_i^2 and μ and σ^2 are estimated from the simultaneous equations

$$S \frac{x_i - \mu}{\sigma^2 + s_i^2} = 0$$

$$S \frac{1}{\sigma^2 + s_i^2} = S \frac{(x_i - \mu)^2}{(\sigma^2 + s_i^2)^2}$$

The solution of these equations is as a rule quite rapid.

In general, a simpler solution will be wanted, and it is worth comparing the efficiencies of the unweighted and weighted means. Consider first the case in which the values of σ^2 and σ_i^2 are known exactly. The variance of the semi-weighted mean

$$S \frac{x_i}{\sigma^2 + s_i^2} / S \frac{1}{\sigma^2 + s_i^2}$$

is
$$1 / S \frac{1}{\sigma^2 + s_i^2}$$

The variance of the unweighted mean is

$$\frac{1}{k} \left(\sigma^2 + \frac{1}{k} S \sigma_i^2 \right)$$

while that of the weighted mean

$$S \frac{x_i}{\sigma_i^2} / S \frac{1}{\sigma_i^2}$$

is
$$S \frac{\sigma^2 + \sigma_i^2}{\sigma_i^4} / S^2 \frac{1}{\sigma_i^2} = S \frac{1}{\sigma_i^2} \left[1 + \sigma^2 \frac{S \frac{1}{\sigma_i^4}}{S \frac{1}{\sigma_i^2}} \right]$$

The relative efficiencies of these three types of mean will depend on the distribution of experimental errors σ_e^2 and on the interaction σ^2 . To obtain some actual figures, the efficiencies have been calculated for the set of experimental variances used in Table II, giving to (λ, μ, ν) the additional values (0.1, 0.8, 0.1) and to (l, m, n) the additional values ($\frac{1}{2}, 1, 3$). This provides nine instead of four examples of variation in experimental errors.

The interaction variance has now to be considered. For each of the nine selected cases, the efficiencies are continuous functions of the interaction variance rv , say. The mean experimental variance for any set $(\lambda, \mu, \nu), (l, m, n)$ is

$$(\lambda l + \mu m + \nu n)v = \kappa v \text{ (say)}$$

and for each of the nine cases, the efficiencies have been calculated for $r = 0, \frac{1}{2}\kappa, \kappa, 2\kappa$, so that the interaction variance is respectively 0, $\frac{1}{2}$, 1, 2 times the mean experimental variance. This gives a $3 \times 3 \times 4$ table of 36 pairs of entries.

TABLE III.

Efficiencies of the Unweighted and Weighted Means.

Proportions in Groups.	Relative Accuracies of the Three Groups of Centres.											
	(1, 1, 2).				(1, 1, 3).				(1, 1, 4).			
	$i=0.$	$i=0.5$	$i=1.$	$i=2.$	$i=0.$	$i=0.5$	$i=1.$	$i=2.$	$i=0.$	$i=0.5$	$i=1.$	$i=2.$
(0.1, 0.8, 0.1)												
U	91	96	98	99	78	91	94	97	67	86	91	95
W	100	98	97	95	100	94	89	85	100	88	81	75
(0.2, 0.6, 0.2)												
U	83	92	96	98	62	84	90	95	48	77	86	93
W	100	97	94	91	100	91	85	79	100	86	78	71
(0.3, 0.4, 0.3)												
U	76	89	94	97	51	78	88	94	36	72	83	92
W	100	96	93	89	100	90	83	76	100	84	75	68

U = Unweighted mean.

W = Weighted mean.

i = Ratio of interaction variance to the mean local error variance.

Several features of the table are obvious. If there is no interaction ($i = 0$), the weighted mean is the same as the efficient solution. This is also the most unfavourable case for the unweighted mean; its efficiency decreases, as the individual experiments diverge more widely in precision, from 91 per cent. to 36 per cent. As the interaction variance increases, the efficiency of the weighted mean falls steadily, while that of the unweighted mean rises steadily. For $i = 0.5$, the weighted mean is still superior to the unweighted, but for $i = 1$, the unweighted mean is superior in all cases, the maximum

loss of information being 17 per cent. in the worst case. For $i = 2$ the loss of information with the unweighted mean is small in all cases.

In practice the situation is much more favourable to the unweighted mean than Table III indicates. For the weights in the semi-weighted mean and in the weighted mean have to be estimated, and the estimation results in a loss of information on these means to which there is no corresponding loss on the unweighted mean. In particular, the information in the weighted mean has been shown above to be decreased in the ratio $\left(\frac{n-4}{n-2}\right)$, where n is the number of degrees of freedom in the local experimental errors. With $n = 16$, for instance, the efficiencies of the weighted means in Table III have to be multiplied by $\frac{9}{7}$. This would make the unweighted mean superior to the mean throughout Table III, except in a few cases in which there was no interaction.

These results indicate that the unweighted mean may safely be recommended where we do not assume that interactions are non-existent, particularly since, with a large number of centres, it is usually necessary to keep the individual experiments small, so that there will rarely be as many as 20 degrees of freedom in the estimates of the local experimental errors.

Where the response varies from centre to centre, it is usually appropriate to test the mean response by comparing it with the variation from centre to centre, especially if the centres constitute a random sample from all possible centres. The usual expression $s_x^2 = S(x - \bar{x})^2 / k(k - 1)$ taken over all centres, is an unbiased estimate of the variance of the unweighted mean \bar{x} . The t -test with $(k - 1)$ degrees of freedom will, however, lead to too many significant results unless the interaction variance is large compared with the local experimental variances, since with one very inaccurate experiment, for instance, the estimate s_x^2 might have a precision based on only one instead of $(k - 1)$ degrees of freedom. The t -test is, however, known to be relatively insensitive to most types of departure from normality in the original data and may be recommended in the great majority of cases, except where the probabilities of a number of tests are being combined, or where the test gives a result very near one of the significance levels and an exact verdict is wanted.

Two alternative tests may prove useful. In many cases it may be sufficiently precise to take account of the signs of the responses only. The efficiency of this test, where the variances are equal, has been shown to be $\frac{2}{\pi}$ or 64 per cent. in (6), where a table of the

5 per cent. points is given. In doubtful cases, with a small number of centres, an exact test of significance may be made by assuming that in the absence of a true mean response, the responses observed at the centres would have occurred with positive or negative signs equally frequently. The complete distribution of the mean may be worked out, as exemplified in (1), pp. 50-4. Bartlett (7) has considered the approximation to this distribution by a continuous frequency distribution, and further work on his lines may enable us to assign an appropriate number of degrees of freedom to s_x^2 in any particular case by use of some statistic such as Fisher's ζ_x ((5), § 14). In a few examples I have worked out, the t -test gives a good approximation.

§ 8. *A Test of Significance of the Variation in the Local Experimental Errors.*

It sometimes happens that the individual experiments may all be regarded as having the same precision, and in this case, as pointed out in § 1, the analysis is much simplified. It is on this account worth having an idea of the amount of purely random sampling variation to be expected in the experimental errors. To obtain this, the experimental variances s_i^2 are each divided by their mean \bar{s}^2 over all k centres. The corresponding values, multiplied by n , should be distributed approximately as χ^2 with n degrees of freedom, and their range may be compared with the published table (5).

A general test of significances of departure from the hypothesis that the variances are all estimates of the same quantity has been given by Neyman and Pearson (8). The test function which they recommend is

$$\prod_{i=1}^k \left(\frac{s_i^2}{\bar{s}^2} \right)^{n_i/2}$$

Tables have been given by Nayer (9).

In conclusion, while the above text has referred verbally to agricultural field experiments, the problems discussed are likely to turn up in any large-scale co-operative experiment. In particular the data considered by Neyman and Pearson (8), which arose in a factory experiment on the control of uniformity of product, are of exactly the same form as those discussed above. Their figures give the breaking strength under tension of small briquettes of cement-mortar. The cement was mixed on each of 10 different days, and 5 briquettes were tested each day. Thus the results provide a mean breaking strength \bar{x}_i for each day and an estimate s_i , based on 4 degrees of freedom, of the variability in the strengths within days. The questions which are of interest in this and similar factory experiments

on control of quality may be somewhat different from those in agricultural field experiments. The question whether the standard errors s_i vary from day to day, which in agricultural field experiments is not of practical importance, except in so far as it affects the efficiency of the experiments, and indeed is usually taken for granted, is one of the prime factors to be tested in a manufacturing experiment. On the other hand, the estimation of the mean of the x_i and the question whether the x_i have varied from day to day or from centre to centre is usually of common interest in both problems, and the discussion given above of the tests of significance may be of use in factory as well as in field experiments.

Summary.

This paper considers the statistical analysis appropriate to experiments which yield, at each of a number of centres or times, an estimate x_i of a treatment effect and an estimate s_i of its standard error, based on n degrees of freedom. This type of data may arise in many modern types of research, as, for instance, series of agricultural field experiments, or factory experiments on the control of quality. The problems considered are the estimation and test of significance of the mean treatment effect and of its variation from centre to centre, these being the most important preliminary questions in agricultural experiments of this type.

If the estimates x_i may be considered equally accurate, *i.e.* if the quantities s_i are all estimates of the same σ , the analysis of variance gives a convenient and familiar method of treatment. Where this is not so, the question is more difficult.

In the absence of any variation in treatment effect from centre to centre, the weighted mean $S\left(\frac{x}{s^2}\right) / S\left(\frac{1}{s^2}\right)$ is suggested as a suitable estimate of the average treatment effect if at least 15 degrees of freedom are available in the estimates s_i . With fewer than 15 degrees of freedom, the weighted mean is not very efficient, and the maximum likelihood estimate, the solution of

$$S \frac{x - \mu}{s^2} + \frac{x - \mu}{(x - \mu)^2} = 0$$

is preferable, since its increased precision, which is equivalent to having three extra degrees of freedom for the estimation of the weights, is well worth the extra labour it involves. With fewer than 6 degrees of freedom in s_i , estimation of the weights involves a considerable loss of information. A comparison is made in this case of the relative efficiencies of the maximum likelihood solution, the unweighted mean, and the weighted mean with an arbitrarily

chosen upper limit to the possible weights, in a set of hypothetical examples designed to cover the variation in experimental errors likely to occur in practice. The weighted mean with fixed upper limit is very satisfactory from the point of view of precision, but the difficulty of assigning a standard error to it is a serious disadvantage. Tests of significance of the ordinary weighted mean, the maximum likelihood solution and the unweighted mean are given.

Where the variation in treatment effect is not assumed non-existent, the unweighted mean should be used. The question of testing its significance by comparison with the variation in response from centre to centre is discussed.

The weighted sum of squares of deviations $Q = S \frac{1}{s^2} (x - \bar{x})^2$ is recommended to test the significance of the variation in treatment effect from centre to centre. An investigation of the frequency distribution of Q is made and a transformation given by which it may be referred to the published table of χ^2 .

Further work is needed to determine more precise tests for the case of a few centres only.

I have to thank Mr. F. Yates for some useful suggestions.

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A CATALOGUE OF UNIFORMITY TRIAL DATA.

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Some Uses of Uniformity Trial Data.

IN a field uniformity trial, the area under experiment is divided into a number of plots, usually all of the same dimensions; the same variety of the crop is grown and the same manurial and cultural operations are carried out on each plot. The yield of each plot is recorded separately at harvest. In some cases other observations are made as well as yield, *e.g.* stand of plants. In the case of tree crops the plot may consist of a single tree or a group of trees.

The usefulness of a uniformity trial lies in the fact that neighbouring units may be amalgamated to form larger plots of various sizes and shapes. The variation in yield over the field due to soil heterogeneity, slight differences in the distribution of manures, errors in weighing, etc. (generally summed up in the term "experimental error"), may be calculated for each type of plot formed. The most obvious use of the data is to provide information on the optimum size and shape of plot, and this is the manner in which the majority of the trials given below have been used. In such studies, once the optimum size and shape have been determined, the standard error per plot and the number of replications required to reach a given degree of accuracy in the comparison of the mean treatment yields are also of interest. This type of information is not, of course, peculiar to uniformity trial data, but is supplied by every properly designed replicated experiment for the particular type of plot used.

A comprehensive study of the uniformity trial data on size and shape of plot has been made by Fairfield Smith,¹ who derives from them an empirical relation of wide applicability between variance per plot and size of plot.

Uniformity trials can also be used to compare the relative efficiencies of different types of experimental design, and, in particular, to test whether any newly proposed design seems suitable for a certain crop. For example, Yates² tested the efficiency of a new method of arranging variety trials on Parker and Batchelor's uniformity data with oranges (catalogue 64). Unfortunately only a small proportion of the trials given below are suitable for comparisons of this kind. For if a trial is intended to provide information on the

optimum size and shape of plot, as most of the trials are, the smallest unit harvested requires to be somewhat smaller than the size of plot likely to be used in practice, so that various shapes of plot may be obtained by amalgamation. In consequence, many trials contain only a few plots of the size which is finally recommended.

The further question whether differences in soil heterogeneity from plot to plot in a field persist year after year is obviously of practical importance. Several trials have been continued on the same site for a number of years, some with the same crop, *e.g.* the trials on Ragi discussed by Lehmann (catalogue 77) and some with varying crops, *e.g.* the Huntley uniform cropping experiment (catalogue 1). As a rule, the yields of the same plot in successive years have been found to be positively correlated, whether the same crop followed or a different crop, but the closeness of the correlation has varied considerably.

The next step was to consider whether these correlations might be used to improve the accuracy of field experiments. With a high correlation it might clearly be worth while to run a uniformity trial as a preliminary to a field trial. The question of how to adjust the yields of the final experiment for differences shown in the uniformity trial at first caused some difficulty. The introduction of the statistical method known as the analysis of covariance, however, provided a means of correction free from any element of arbitrariness, and gave a stimulus to studies on the value of a uniformity trial as a preliminary to field experimentation. The results of these investigations are now well known. With annual agricultural crops, uniformity trials have not in general doubled the precision of subsequent field trials, whereas they entail approximately double the labour of a field trial with no previous uniformity trial, and a year's delay in the experimental results. With perennial plants, such as rubber, for example, where each plot consists of the same trees or bushes year after year, the gain in precision is decidedly higher, and preliminary records may often be obtained without much extra labour, or may indeed be part of a standard observational programme. The case for a preliminary uniformity trial is then considerably stronger. In animal nutrition work, also, the experimental unit is the same in the uniformity trial and the actual experiment, and the covariance method has proved strikingly successful in some such cases (*cf.* Bartlett).³

Uniformity trial data have also occasionally been used as a check on the applicability to field experiments of the analysis of variance and the tests of significance based on it. The mathematical theory from which the *z* table is derived requires the assumptions that the experimental yields are normally distributed and that their deviations

from the means about which they vary are uncorrelated. These assumptions are known to be untrue for field trials. A preliminary requirement for the application of the analysis of variance to be possible is that the experimental design used should be chosen at random from a set of designs such that, in the absence of any treatment effect, the average treatment mean square over the set should equal the average error mean square. The repeated use of the same design, however excellent in itself, is condemned on these grounds, and Tedin ⁴ has estimated the bias in the Knut Vik square from a set of uniformity trial data. The further question arises: how good an approximation to the tabulated z distribution is generated by the process of randomization used? There again the question may be tested from uniformity trial data. One such example has been worked out by Eden and Yates,⁵ and further examples would be of considerable interest.

The large number of uniformity trials which have been carried out and the applications mentioned above testify that uniformity trial data play an important part in modern research on field technique. A catalogue of the uniformity trial data at present available therefore appears likely to be of value, in order to facilitate further research in field technique, and also to bring to light unpublished material which might otherwise be lost. With this end in view, we have at Rothamsted during the last few years been constructing a card index of such trials, and we have also encouraged workers with whom we have come into contact and who have conducted uniformity trials, but who have for various reasons been unable to examine the results, or who have examined them but have not published their conclusions, to file a copy of their material at Rothamsted. The following workers have furnished us with material of this nature:

Worker.	Crop.	No. and size of plots, etc.	Total area.	Catalogue No.
G. H. Goulden...	Barley	2304 plots 3' × 3'	$\frac{1}{2}$ acre	8
F. J. Pound ...	Cacao	Several thousand trees since 1914	—	14
S. M. Gilbert ...	Coffee	12,000 tree yields of cherry	—	18
H. C. Ducker ...	Cotton	490 plots 1 row × 21'	$\frac{1}{2}$ acre	26
O. V. S. Heath ...	Cotton	3696 individual plants	—	28
D. MacDonald ...	Cotton	1152 plots 3 $\frac{1}{2}$ ' × 30' or 40'	3 $\frac{1}{2}$ acres	30
A. H. McKinstry	Cotton	480 plots 1 row × 25'	1 acre	31
A. R. Saunders	Maize	250 plots 1 row × 10 plants	—	40
Huntley (Mon.)	Oats	46 plots 23 $\frac{1}{2}$ ' × 317'	8 acres	55
J. Grantham *	Rubber	1000 trees for 10 years	—	85
H. Evans	Sugar			
	Cane	710 plots 5' × 50'	6 acres	106
H. F. Smith ...	Wheat	1080 plots 6" × 1'	$\frac{1}{2}$ acre	130

* Grantham's data on rubber have already been utilized by Murray in the paper referred to in the catalogue.

In most cases in which uniformity trials have been used as the basis of published work, but in which the original data have not been published, we have written to the authors concerned suggesting that they might like to file copies of these data at Rothamsted, so as to make it accessible to other workers. This suggestion has been met for nineteen trials.

Finally, in seven cases the data, although not published, are known to have been filed elsewhere.

The entries in the catalogue can therefore be classified as follows :

Material.	No of entries in catalogue.		No. of trials.		Average no. of plots per trial.	
	Field crops.	Trees.	Field crops.	Trees.	Field crops.	Trees.
Already published ...	73	15	135	25	221	225
Not published ...	14	1	22	1	554	500
Not published but copy filed
{ at Rothamsted ...	21	4	28	4	539	3,761
{ elsewhere ...	5	1	6	1	1,440 *	50

* One entry contains 203 sugar-cane trials each of 36 plots. This entry has been omitted when finding the average number of plots per trial.

As is to be expected from considerations of space, the average number of plots per trial is considerably greater for trials the yields of which have not been published than for those which have. This makes the recovery of such data the more valuable.

It must not be assumed that in the 14 entries given as "not published" the data are inaccessible. In some cases we have not been able to get into touch with the author, perhaps owing to change of address, and in others replies have not yet been received, but we hope in time to reduce this, and students are meanwhile advised to write to the author concerned about such trials.

This catalogue will not have been in vain if it has rescued from oblivion the 32 uniformity trials now filed at Rothamsted. It will be more valuable if, as we hope, it encourages other workers at present unknown to us, who have carried out uniformity trials, to furnish particulars of these, and if possible to make available copies of the original data. We should also be grateful for any information on omissions from this list. Although it is, we hope, fairly comprehensive as regards English (including Empire) and American Journals, we make no pretence to have searched the Continental literature at all thoroughly. This task we commend to some other worker.

In conclusion, we must thank the workers who helped in the compilation by sending data or information.

References.

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- ² F. Yates, *ibid.*, 1936, Vol. 26, pp. 424-55.
- ³ M. S. Bartlett, *ibid.*, 1935, Vol. 25, pp. 238-44.
- ⁴ O. Tedin, *ibid.*, 1931, Vol. 21, pp. 191-208.
- ⁵ T. Eden and F. Yates, *ibid.*, 1933, Vol. 23, pp. 6-17.

The Catalogue.

The entries are arranged alphabetically under crops, and for each crop alphabetically under author's names. The information given is as follows: the size and shape of the smallest unit harvested, its approximate area as a fraction of an acre, and the approximate total area (T.A.) occupied by the trial. In some cases complete information on these points was not available.

The following symbols have been used to show where the data may be found:—

Published in the paper	G
Not published	N
Not published, but filed:					
at Rothamsted	R
Elsewhere	E

Notes have occasionally been added to the entries in cases where several measurements were made on the crop.

- Alfalfa. 1. 46 plots, each $23\frac{1}{2}' \times 317' = \frac{1}{8}$ a. T.A. 8 a. G.
 (1) 1912-14. Harris, J. A., and Scofield, C. S. Permanence of differences in the plats of an experimental field. *J. Agric. Res.*, **20**, 335-56.
 (2) 1922-23-24.
 Further studies on the permanence of differences in the plats of an experimental field. *J. Agric. Res.*, **36**, 15-40.
2. 36 plots, each $\frac{1}{10}$ a. T.A. 2 a. R.
 3 years 1930-31-32.
 Metzger, W. H. The relation of varying rainfall to soil heterogeneity as measured by crop production. *J. Amer. Soc. Agron.*, **27**, 274-78.
3. 175 plots, each $13\cdot2' \times 13\cdot2' = \frac{1}{20}$ a. G.
 T.A. $\frac{3}{4}$ a.
 Summerby, R. The value of preliminary uniformity trials in increasing the precision of field experiments. *Macdonald Coll. Tech. Bull.* 15.

- Apples.
4. 512 individual tree yields. G.
Batchelor, L. D., and Reed, H. S. Relation of the variability of yields of fruit trees to the accuracy of field trials. *J. Agric. Res.*, 12, 245-83.
 5. 50 individual tree yields. E.
Collison, R. C., and Harlan, J. P. Variability and size relations in apple trees. *New York (Geneva), Agr. Exp. Sta. Tech. Bull.* 164, 1-38.
Yields filed at the New York State Agricultural Experiment Station, Geneva, N.Y.
 6. 187 individual tree yields. G.
Strickland, A. G. Error in horticultural experiments. *J. Dept. Agric. Victoria*, 1935, 32, 408-16.
Time in weeks for a stored apple to reach 5 per cent. waste and 5 per cent. breakdown.
- Barley.
7. 390 plots, each $4' \times 4' = \frac{1}{3000}$ a. T.A. $\frac{1}{8}$ a. G.
Bose, R. D. Some soil heterogeneity trials at Pusa and the size and shape of experimental plots. *Ind. J. Agric. Sci.*, 5, 545.
 8. 2304 plots, each $3' \times 3' = \frac{1}{4000}$ a. T.A. $\frac{1}{2}$ a. R.
Goulden, C. H. Unpublished data.
 9. (1) 30 plots, each $\frac{1}{1000}$ a. T.A. $\frac{1}{2}$ a. G.
(2) 128 plots, each $\frac{1}{1000}$ a. T.A. 1 a.
Hanson, N. A. Prøvedyrkning paa Forsøgsstationen ved Aarslev. *Tids. for Landbrugets Planteavl.*, 21, 553.
 10. 46 plots, each $23\frac{1}{2}' \times 317' = \frac{1}{8}$ a. T.A. 8 a. G.
(1) 1912-14, Harris, J. A., and Scofield, C. S. Permanence of differences in the plats of an experimental field. *J. Agric. Res.*, 20, 335-56.
(2) 1922-23-24.
Further studies on the permanence of differences in the plats of an experimental field. *J. Agric. Res.*, 36, 15-40.
 11. 234 plots, each $24\frac{1}{2}' \times 34\frac{1}{2}' = \frac{1}{800}$ a. T.A. $4\frac{1}{2}$ a. G.
Kristensen, R. K. Anlæg og Opgrelsa af Marksforssq. *Tids. for Landbrugets Planteavl.*, 31.

12. 96 plots, each $3\cdot3' \times 3\cdot3' = \frac{1}{4} \frac{1}{1000}$ a. T.A. $\frac{1}{10}$ a. G.
N contents given, but not yields.
Barbacki, St. *Mémoires de l'Institut National Polonais d'Economie Rural. à Putawy*, T. XIV, No. 213.
- Cacao. 13. 500 trees : yields in pods. N.
Cheesman, E. E., and Pound, F. J. Uniformity trials on Cacao. *Trop. Agric.*, 9, 277-88.
14. Pound, F. J. Unpublished data of several thousand trees since 1914. R.
- Clover. 15. 35 plots, each $13\cdot2' \times 66' = \frac{1}{80}$ a. T.A. $\frac{3}{4}$ a. G.
Each year 1928-32 on different parts of the same field.
Summerby, R. The value of preliminary uniformity trials in increasing the precision of field experiments.
- Coconuts. 16. 60 plots, each of 6 trees. T.A. 12 a. G.
Joachim, A. W. R. A uniformity trial with coconuts. *Tropical Agriculturist*, 85, 4, 198-207.
Yields of nuts over 8 months.
17. 44 plots, each of 25 palms.
Yields each year from 1919 to 1928.
Beckett, W. H. R.
Randomization in Field Experiment and its application on experiment stations. *Bull. No. 20, Dept. of Agric., Gold Coast*, number of nuts given.
- Coffee. 18. 12,000 individual tree yields of cherry for each R.
of 3 years. Gilbert, S. M. Unpublished data.
- Corn. 19. 3 trials, 2304 plots, each 1 hill \times 1 row (1) 1923, (2) 1925, (3) 1925. T.A. $\frac{3}{4}$ a. E.
Bryan, A. A. Factors affecting experimental error in field plot tests with corn. *Iowa Agric. Expt. Sta. Report*, 1930-31, 67.
Individual yields filed with Iowa Agric. Exp. Sta.
20. 450 plots, each $21' \times 68'$ ($3\frac{1}{2}'$ discard all round) $= \frac{1}{81}$ a. T.A. 9 a. G.
Garber, R. J., McIlvaine, J. C., and Hoover, M. M. A method of laying out experimental plats. *J. Amer. Soc. Agron.*, 23, 286-98.

21. 46 plots, each $23\frac{1}{2}' \times 317' = \frac{1}{8}$ a. T.A. 8 a. G.
1915-16.

Harris, J. A., and Scofield, C. S.
Permanence of differences in the plats of an
experimental field. *J. Agric. Res.*, 20, 335-56.

22. 36 plots, each $\frac{1}{20}$ a. T.A. $1\frac{1}{2}$ a. R.

Metzger, W. H. The relation of varying rain-
fall to soil heterogeneity as measured by crop
production. *J. Amer. Soc. Agron.*, 27, 274-78.

23. 438 plots, each 1 row $\times 66' = \frac{1}{81}$ a. T.A.
 $2\frac{1}{2}$ a. G.

McClelland, C. K. Some determinations of
plot variability. *J. Amer. Soc. Agron.*, 18,
819-23.

24. 120 plots, each $\frac{1}{10}$ a. T.A. 12 a. G.

Smith, L. H. Plot arrangement for variety
experiments with corn. *Proc. Amer. Soc.
Agron.*, 1, 84-89.

Cotton.

25. 5 trials, each of about 160 plots, each 20
ridges $\times 7$ metres $= \frac{1}{6}$ a. T.A. 4 a. G.

Bailey, M. A., and Trought, T. An account
of experiments carried out to determine the
experimental error of field trials with cotton
in Egypt. *Min. Agric. Egypt Tech. and Sc.
Service Bull.* 63.

26. 490 plots, each 1 row $\times 21' = \frac{1}{100}$ a. T.A.
 $\frac{3}{4}$ a. R.

Ducker, H. C. Unpublished data.

27. (1) 200 plots, each $1' \times 24' = \frac{1}{100}$ a.
T.A. $\frac{1}{10}$ a. N.

(2) 200 plots, each $4\frac{1}{2}' \times 16' = \frac{1}{100}$ a.
T.A. $\frac{1}{3}$ a.

Fu Siao. Uniformity trials with cotton. *J.
Amer. Soc. Agron.*, 27, 12.

28. 3696 individual plants. R.

Heath, O. V. S. Unpublished data.

Height, node number and dry matter of
individual cotton plants.

29. 1280 plots, each 4 rows $\times 4.8' = \frac{1}{100}$ a.
T.A. $\frac{3}{4}$ a. R.

Hutchinson, J. B., and Panse, V. G.
Studies in the technique of field experiments.
I. *Indian J. Agric. Sci.*, 5, 523-38.

30. (1) 576 plots, each $3.5' \times 40' = \frac{1}{800}$ a. T.A. 2 a.
 (2) 576 plots, each $3.5' \times 30' = \frac{1}{400}$ a. T.A. $1\frac{1}{2}$ a. R.
 MacDonald, D. Unpublished data.
31. 480 plots, each 1 row $\times 25' = \frac{1}{800}$ a. T.A. 1 a. R.
 McKinstry, A. H. Unpublished data.
32. (1) 300 plots, each $3' \times 48' = \frac{1}{800}$ a. T.A. 1 a. R.
 (2) 700 plots, each $3\frac{1}{2}' \times 47' = \frac{1}{800}$ a. T.A. $2\frac{1}{2}$ a.
 Reynolds, E. B., Killough, D. T., and Vantine, J. T.
 Size, shape and replication of plats for field experiments with cotton. *J. Amer. Soc. Agron.*, 26, 725-34.
- Fodder 33. 63 plots, each $15' \times 112\frac{1}{2}' = \frac{1}{26}$ a. T.A. $2\frac{1}{2}$ a. G.
 Corn. Morgan, J. O. Some experiments to determine the uniformity of certain plats for field tests. *Proc. Amer. Soc. Agron.*, 1, 58-67.
- Grapes. 34. 200 vines, 8' apart in rows 10' apart. G.
 Strickland, A. G., Forster, H. C., and Vasey, A. J. A vine uniformity trial. *J. Agric. of Victoria*, 30, 584.
- Hops. 35. 30 plots, each 1 row $\times 210'$. Yields each year. 1909-14. G.
 Stockberger, W. W. Relative precision of formulæ for calculating normal plot yields. *J. Amer. Soc. Agron.*, 8, 167-75.
- Lemons. 36. 364 individual tree yields. G.
 Batchelor, L. D., and Reed, H. S. Relation of the variability of yields of fruit trees to the accuracy of field trials. *J. Agric. Res.*, 12, 245-83.
- Lentils. 37. 390 plots, each $4' \times 4' = \frac{1}{2250}$ a. T.A. $\frac{1}{4}$ a. G.
 Bose, R. D. Some soil heterogeneity trials at Pusa and the size and shape of experimental plots. *Ind. J. Agric. Sci.*, 5, 545.
- Maize. 38. 83 plots, each $33' \times 33' = \frac{1}{40}$ a. T.A. $2\frac{1}{8}$ a. G.
 Beckett, W. H., and Fletcher, S. R. B. A uniformity trial with maize. *Gold Coast Dept. Agric. Bull.* 16, 222-26.
 Germination and ear number counts given.
 Yields measured for 15 plots only.

39. 300 plots, each 1 row \times 60'. R.
Saunders, A. R. Statistical methods with special reference to field experiments. *Union of South Africa, Dept. of Agric. and Forestry, Science Bull.* 147.
40. 250 plots, each 1 row \times 10 plants. R.
Saunders, A. R. Unpublished data.
41. (1) 175 plots each $13.2' \times 13.2' = \frac{1}{2} \frac{1}{80}$ a. G.
T.A. $\frac{3}{4}$ a.
Yields each year from 1922-26.
(2) 35 plots, each $13.2' \times 66' = \frac{1}{80}$ a.
T.A. $\frac{3}{4}$ a.
Yields each year from different ranges 1927, 1928, 1929, 1930, 1931, 1932.
Summerby R. The value of preliminary uniformity trials in increasing the precision of field experiments.
- Mangolds. 42. 30 plots, each $\frac{1}{160}$ a. T.A. $\frac{1}{2}$ a. G.
Hanson, N. A. Prøvedyrkning paa Forsøgsstationen ved Aarslev. *Tids. for Landbrugets Planteavl.*, 21, 553.
43. 200 plots, each 3 rows \times $30\frac{1}{4}' = \frac{1}{160}$ a. T.A. 1 a. G.
Mercer, W. B., and Hall, A. D. The experimental error of field trials. *J. Agric. Sci.*, 4, 107-132.
44. (1) 175 plots, each $13.2' \times 13.2' = \frac{1}{2} \frac{1}{80}$ a. G.
T.A. $\frac{3}{4}$ a.
(2) 150 plots, each $13.2' \times 13.2' = \frac{1}{2} \frac{1}{80}$ a.
T.A. $\frac{1}{2}$ a.
Summerby, R. The value of preliminary uniformity trials in increasing the precision of field experiments.
45. 1050 plots, each $\frac{1}{1600}$ a. T.A. 1 a. N.
Wood, T. B., and Stratton, F. J. M. The interpretation of experimental results. *J. Agric. Sci.*, 3, 417-40.
- Millet. 46. 105 plots, each $\frac{1}{200}$ a. T.A. $\frac{1}{2}$ a. G.
Lehmann, A. *Report of Agricultural Chemist. Dept. of Agric. Mysore State, 1900-7.* Roemer, Th. *Der Feldversuch. Arbeiten der Deutschen Landw. Gesellschaft.*, 302.

47. 600 plots, each $1' \times 15' = \frac{1}{3000}$ a. T.A. $\frac{1}{4}$ a. G.
 Li, H. W., Meng, C. J., and Liu, T. N.
 Field results in a millet-breeding experiment.

- Mushrooms. 48. (1) 50 plots each $2' \times 5' = \frac{1}{4800}$ a. T.A. $\frac{1}{100}$ a. G.
 (2) 50 plots each $4' \times 6' = \frac{1}{1800}$ a. T.A.
 $\frac{1}{40}$ a.
 (3) 40 plots each $4' \times 6' = \frac{1}{1800}$ a. T.A.
 $\frac{1}{50}$ a.

Lambert, E. B. Size and arrangement of plots for yield tests with cultivated mushrooms. *J. Agric. Res.*, **43**, 1971-80.

- Oats. 49. (1) 66 plots, each $\frac{1}{4}$ a. T.A. 17 a. R.
 (2) 68 plots, each $\frac{1}{4}$ a. T.A. 17 a.
 Farrell, F. D. Interpreting the variability of plat yields. *U.S. Dept. of Agric. Bureau of Plant Industry Circular No. 109*, 27-32.
 50. 295 plots, each $21' \times 68' = \frac{1}{81}$ a. T.A. 6 a. G.
 Garber, R. J., McIlvaine, T. C., and Hoover, M. M. A study in soil heterogeneity in experiment plots. *J. Agric. Res.*, **33**, 255-68.
 51. 450 plots, each $21' \times 68'$ ($3\frac{1}{2}$ feet discard all round) = $\frac{1}{81}$ a. T.A. 9 a. G.
 Garber, R. J., McIlvaine, T. C., and Hoover, M. M. A method of laying out experimental plots. *J. Amer. Soc. Agron.*, **23**, 286-98.
 52. (1) 200 plots (3 yields missing) each = $\frac{1}{800}$ a. G.
 (2) 300 plots (3 yields missing) each = $\frac{1}{600}$ a.
 Gorski, M., and Stefaniow, M. Die Anwendbarkeit der Wahrscheinlichkeitsrechnung bei Feldversuchen. *Landw. Versuchsstationen*, **90**, 225-40.
 53. (1) 30 plots, each $\frac{1}{100}$ a. T.A. $\frac{1}{2}$ a. G.
 (2) 128 plots, each $\frac{1}{100}$ a. T.A. 1 a.
 Hanson, N. A. Prøvedyrkning paa Forsøgstationen ved Aarslev. *Tids. for Landbrugets Planteavl.*, **21**, 553.
 54. 46 plots, each $23\frac{1}{2}' \times 317' = \frac{1}{6}$ a. T.A. 8 a. G.
 1917. Harris, J. A., and Scofield, C. S. Permanence of differences in the plats of an experimental field. *J. Agric. Res.*, **20**, 335-56.
 55. 46 plots, each $23\frac{1}{2}' \times 317' = \frac{1}{6}$ a. T.A. 8 a. R.
 Same trial as No. 54. 1911, total produce only.

56. 207 plots, each $\frac{1}{30}$ a. T.A. 7 a. G.
 Kiesselbach, T. A. Studies concerning the elimination of experimental error in comparative crop tests. *Res. Bull. Nebraska Agric. Stat.*, 13, 1-95.
57. 36 plots, each $\frac{1}{20}$ a. T.A. $1\frac{1}{2}$ a. R.
 Metzger, W. H. The relation of varying rainfall to soil heterogeneity as measured by crop. *J. Amer. Soc. Agron.*, 27, 274.
58. 24 plots, each $33' \times 132' = \frac{1}{10}$ a. T.A. $2\frac{1}{2}$ a. G.
 McClelland, C. K. Some determinations of plot variability. *J. Amer. Soc. Agron.*, 18, 819-23.
59. 240 plots, each $\frac{1}{1200}$ a. T.A. $\frac{1}{4}$ a. G.
 Roemer, Th. Der Feldversuch. *Arbeiten der deutschen Landw. Gesellschaft*, 302.
60. 48 plots, each $\frac{1}{10}$ a. T.A. 5 a. G.
 Roth. *Exp. Sta. Report*, 1927-28, p. 153.
61. 512 plots, each $1' \times 15' = \frac{1}{3000}$ a. T.A. $\frac{1}{4}$ a. G.
 Summerby, R. A study of size of plats, numbers of replications, and the frequency and methods of using cheek plats in relation to accuracy in field experiments. *J. Amer. Soc. Agron.*, 17, 140-50.
62. (1) 175 plots, each $13.2' \times 13.2' = \frac{1}{80}$ a. T.A. $\frac{3}{4}$ a. G.
 Yields each year 1922-26 and 1924-25-26.
 (2) 35 plots, each $13.2' \times 66' = \frac{1}{80}$ a. T.A. $\frac{3}{4}$ a. Each year on different ranges from 1927 to 1932.
 Summerby, R. The value of preliminary uniformity trials in increasing the precision of field experiments.
63. 124 plots, each $33' \times 132' = \frac{1}{10}$ a. T.A. $12\frac{1}{2}$ a. G.
 Wyatt, F. A. Variation in plot yields due to soil heterogeneity. *Sci. Agr.*, 7, 248-56.

Oranges.

64. (1) 1000 individual tree yields. G.
 (2) 495 individual tree yields.
 (3) 240 individual tree yields.
 Batchelor, L. D., and Reed, H. S. Relation of the variability of yields of fruit trees to the accuracy of field trials. *J. Agric. Res.*, 12, 245-83.

65. 193 plots, each of 8 trees, yields given each year from 1921 to 1927. G.
 Parker, E. D., and Batchelor, L. D. Variation in the yields of fruit trees in relation to the planning of future experiments. *Hilgardia* 7, No. 2, 1932.
- Paddy. 66. (1) 104 plots, each $6.6' \times 122' = \frac{1}{84}$ a. T.A. N.
 2 a.
 (2) 72 plots, each $6.6' \times 174' = \frac{1}{38}$ a. T.A.
 2 a.
 Lord, L. Irrigated paddy: a contribution to the study of field plot technique. *Agric. J. India*, 19, 20-27.
- Pasture. 67. 760 plots, each $6.6' \times 3.3' = \frac{1}{2000}$ a. T.A. $\frac{1}{2}$ a. G.
 Davies, J. G. The experimental error of the yield from small plots of natural pasture. *Council Sci. and Indust. Res. (Aust.) Bull.* 48.
- Peaches. 68. 144 individual tree yields. G.
 Strickland, A. G. Error in horticultural experiments. *J. Dept. Agric. Victoria*, 33, 408-16.
- Pineapples. 69. (1) 24 plots, each 4 rows \times 75'. G.
 (2) 24 plots, each 4 rows \times 75'.
 (3) 25 plots, each 4 rows \times 60'.
 Magistad, O. C., and Farden, C. A. Experimental error in field experiments with pineapples. *J. Amer. Soc. Agron.*, 26, 631-44.
- Potatoes. 70. 750 single-row plots. E.
 Jakowski, Z. Unpublished data, see Neyman, J. Statistical problems in agricultural experimentation. *J. Roy. Stat. Soc. Suppl.*, 2, 107-54.
 Yields filed with J. Neyman.
71. 618 plots, each $2.2' \times 33.5' = \frac{1}{800}$ a. T.A. 1 a. E.
 Justesen, S. H. Influence of size and shape of plots on the precision of field experiments with potatoes. *J. Agric. Sci.*, 22, 366-72.
 Data filed with the N.I.A.B., Cambridge, England.
72. 576 plots, each $3' \times 22' = \frac{1}{700}$ a. T.A. 1 a. G.
 Kalamkar, R. J. Experimental error and the field plot technique with potatoes. *J. Agric. Sci.*, 22, 373-85.

73. 204 plots, each $2\frac{1}{2}' \times 72\frac{1}{2}' = \frac{1}{3}\frac{1}{10}$ a. T.A. 1 a. G.
 Lyon, T. L. Some experiments to estimate errors in field plat tests. *Proc. Amer. Soc. Agron.*, 3, 89-114.
74. 720 plants, every fifth hill missing. G.
 Stewart, F. Missing hills in potato fields: their effect upon the yield. *New York Agric. Exp. Sta. Bull.* 459, 45-69.
75. 4 sets, each $3' \times 15' = \frac{1}{1000}$ a. N.
 (1) 1000 plats. (2) 1560 plats. (3) 2000 plats. (4) 1000 plats.
 Thompson, R. C. Size, shape, etc., in sweet potatoes field-plot experiments. *J. Agric. Res.*, 48, 379-99.
76. 51 plots, each $\frac{1}{20}$ a. T.A. $2\frac{1}{2}$ a. N.
 Westover, K. C. The influence of plat size and replication on experimental errors in field trials with potatoes. *West. Virginia. Agr. Expt. Sta. Bull.* 189.
- Ragi. 77. 34 plots, each $\frac{1}{200}$ a. T.A. $\frac{1}{8}$ a. G.
 Yields for 4 years 1905-8.
 Lehmann, A. *Report of Agric. Chemist. Dept. of Agric. Mysore State, 1900-7.* See also Roemer, *Der Feldversuch.* 1st Ed.
- Rice. 78. (1) 144 plots each $5' \times 5' = \frac{1}{1740}$ a. T.A. $\frac{1}{12}$ a. N.
 (2) 144 plots each $5' \times 5' = \frac{1}{1740}$ a. T.A. $\frac{1}{12}$ a.
 Plots arranged in a 12×12 Latin square.
 Bose, S. S., Ganguli, P. M., and Mahalanobis, P. C. The frequency distribution of plot yields and the optimum size of plots in a uniformity trial with rice in Assam. *Indian J. Agric. Sci.*, 1936, 6 part 5, pp. 1107-22.
79. 3 series of 100 plots, each $1\frac{1}{2}' \times 14.2' = \frac{1}{2000}$ a. T.A. $\frac{1}{20}$ a. N.
 Chien-Liang-Pan. Uniformity trials with rice. *J. Amer. Soc. Agron.*, 27, 279.
80. 54 plots each $33' \times 33' = \frac{1}{40}$ a. T.A. $1\frac{1}{4}$ a. G.
 Coombs, G. E., and Grantham, J. Field experiments and the interpretation of their results. *Agr. Bull. Fed. Malay States*, 4.

81. 300 plots, each $1.5' \times 14.25' = \frac{1}{2000}$ a. T.A. $\frac{1}{2}$ a. N.
 Li-Ying-Shen. Statistical analysis of a blank test of rice with suggestions for field technique. *Agricultura Sinica*, 1934, 1 No. 4, pp. 107-50.
82. 560 plots, each $10' \times 10' = \frac{1}{480}$ a. T.A. $1\frac{1}{2}$ a. G.
 Lord, L. A uniformity trial with irrigated broadcast rice. *J. Agric. Sci.*, 21, 178-86.
83. Plots $3' \times 3' = \frac{1}{840}$ a. N.
 Mitra, S. H., and Ganguli, P. M. A uniformity trial in rice. *Proc. 21st Annual Indian Sci. Congress Bombay*, 1934, 71.
84. 280 plots, each 2 rows \times 10 plants. N.
 Parnell, F. R. Experimental error in variety tests with rice. *Agric. J. India*, 14, 747-57.
 See also No. 66.
- Rubber. 85. 1000 trees yields for each of 10 years. R.
 Murray, R. K. S. The value of a uniformity trial in field experimentation with rubber. *J. Agric. Sci.*, 24, 177-84.
86. 161 trees each year from 1921-22 to 1924-25. G.
 Taylor, R. A. The inter-relationship of yield and the various vegetative characters in *Hevea Brasiliensis*. *Dept. of Agric. Ceylon Bull.* 77.
- Rye. 87. (1) 30 plots, each $\frac{1}{60}$ a. T.A. $\frac{1}{2}$ a. G.
 (2) 128 plots, each $\frac{1}{60}$ a. T.A. 1 a.
 Hanson, N. A. Prøvedyrkning paa Forsøgsstationen ved Aarslev. *Tids. for Landbrugets Planteavl.*, 21, 553.
- Seeds. 88. 128 plots, each $\frac{1}{60}$ a. T.A. 1 a. G.
 Hanson, N. A. Prøvedyrkning paa Forsøgsstationen ved Aarslev. *Tids. for Landbrugets Planteavl.*, 21, 553.
- Silage corn. 89. 46 plots, each $23\frac{1}{2}' \times 317' = \frac{1}{4}$ a. T.A. 8 a. G.
 1918. Harris, J. A., and Scofield, C. S. Permanence of differences in the plats of an experimental field. *J. Agric. Res.*, 20, 335-56.
 1920, 1925.
 Further studies on the permanence of differences in the plats of an experimental field. *J. Agric. Res.*, 36, 15-40.

- Sorghum.** 90. 160 plots, each $\frac{1}{100}$ a. for 1930–31–32. T.A. 1 a. G.
Kulkarni, R. K., Bose, S. S., and Mahalanobis, P. C. The influence of shape and size of plots on the effective precision of field experiments with sorghum. *Indian J. Agric. Sci.*, 6, 460–74.
91. 2000 plots, each 1 row \times 1 rod = $\frac{1}{100}$ a. T.A. $2\frac{1}{2}$ a. G.
Stephens, J. C., and Vinall, H. N. Experimental methods and the probable error in field experiments with sorghum. *J. Agric. Res.*, 37, 629–46.
92. 400 plots, each $3\cdot3' \times 33' = \frac{1}{100}$ a. T.A. 1 a. N.
Swanson, A. F. Variability of grain sorghum yields as influenced by size, shape and number of plats. *J. Amer. Soc. Agron.*, 22, 833–38.
- Sorgo.** 93. 36 plots, each $\frac{1}{100}$ a. T.A. $1\frac{3}{4}$ a. R.
2 years, 1932–33.
Metzger, W. H. The relation of varying rainfall to soil heterogeneity as measured by crop production. *J. Amer. Soc. Agron.*, 27, 274–78.
- Soy beans.** 94. 30 plots: artificially constructed in frames, each $4\frac{3}{4}' \times 9\frac{1}{2}' = \frac{1}{100}$ a. T.A. $\frac{3}{10}$ a. G.
Garber, R. J., and Pierre, W. H. Variation of yields obtained in small artificially constructed field plats. *J. Amer. Soc. Agron.*, 25, 98–105.
95. (1) 882 plots, each 1 row \times 8' = $\frac{1}{100}$ a. T.A. $\frac{1}{2}$ a. G.
(2) 1540 plots, each 1 row \times 8' = $\frac{1}{100}$ a. T.A. $\frac{3}{4}$ a.
Odland, T. E., and Garber, R. J. Size of plot and number of replications in field experiments with soy beans. *J. Amer. Soc. Agron.*, 20, 94–108.
- Strawberries.** 96. (1) 120 plots, each $4' \times 68' = \frac{1}{100}$ a. T.A. $\frac{3}{4}$ a. N.
(2) 80 plots, each $4' \times 34' = \frac{1}{200}$ a. T.A. $\frac{1}{4}$ a.
Wilcox, A. N. A study of field plot technique with strawberries. *Scientific Agriculture*, 8, 171–74.

- Sugar-beet.** 97. 46 plots, each $23\frac{1}{2}' \times 317' = \frac{1}{8}$ a. T.A. 8 a. G.
Harris, J. A., and Scofield, C. S. Permanence of differences in the plots of an experimental field. *J. Agric. Res.*, **20**, 335-56.
98. 600 plots, each 1 row $\times 33' = \frac{1}{700}$ a. T.A. 1 a. G.
Immer, F. R. Size and shape of plots in relation to field experiments with sugar-beets. *J. Agric. Res.*, **44**, 649-68.
99. 600 plots, each 1 row $\times 33' = \frac{1}{700}$ a. T.A. 1 a. R.
Immer, F. R., and Raleigh, S. M. Further studies of size and shape of plot in relation to field experiments with sugar-beet. *J. Agric. Res.*, **47**, 591-98.
100. 416 plots, each $8' \times 135' = \frac{1}{16}$ a. T.A. $10\frac{1}{2}$ a. G.
Roemer, Th. Der Feldversuch. *Arbeiten der deutschen Landw. Gesellschaft*, **302**.
101. 96 plots, each 1 row $\times 55\cdot8'$. G.
Two sets, 1916 and 1918.
Roemer, Th. Der Feldversuch. *Arbeiten der deutschen Landw. Gesellschaft*, **302**.
- Sugar cane.** 102. 49 plots, each $\frac{1}{80}$ a. T.A. 1 a. R.
Barbados, 1927.
103. 48 plots, each $30' \times 75' = \frac{1}{16}$ a. T.A. $2\frac{1}{2}$ a. G.
Borden, R. J. Replications of plot treatments in field experiments. *Hawaiian Planters' Record*, **34**, 151-55.
104. 203 trials each of 36 plots. E.
Demandt, E. Die Resultaten der Blanco-Proeven met 2878 PoJ van Oogstjaar 1931. Archief voor de Suikerindustrie in Nederlandsch-Indië Deel III. *Med. van het Proefstation voor Java Suikerindustrie Jahrgang*, 1932, 14.
Yields filed at the Proefstation voor de Java. Suikerindustrie, Soerabaia, Java.
105. Yields of 1200 individual stools. G.
Evans, H. Some preliminary data concerning the best shape and size of plot for field experiments with sugar cane. *Dept. of Agric. Mauritius. Sugar Cane Research Station Bull.* 3.
106. 710 plots, each $5' \times 50' = \frac{1}{176}$ a. T.A. 6 a. R.
H. Evans. Unpublished data.

107. (1) 960 plots, each $3' \times 30\frac{1}{2}' = \frac{1}{8}\frac{1}{16}$ a. T.A. $1\frac{1}{2}$ a. R.
 (2) 1088 plots, each $3' \times 60' = \frac{1}{2}\frac{1}{2}$ a. T.A. $4\frac{1}{2}$ a. G.
 Wynne Sayer, Vaidyanathan and Subramaria Iyer. Ideal size and shape of sugar-cane experimental plots based upon tonnage experiments with Co 205 and Co 213 conducted in Pusa. *Indian J. Agric. Sci.*, 1936, 6.
108. 968 plots, each $3' \times 60' = \frac{1}{2}\frac{1}{2}$ a. T.A. 4 a. G.
 Wynne Sayer and Krishna Iyer. On some of the factors that influence the error of field experiments with special reference to sugar cane. *Indian J. Agric. Sci.*, 1936, 6, 917.
- Swedes. 109. 48 plots, each $\frac{1}{16}$ a. T.A. 5 a. G.
Roth. Exp. Sta. Report, 1925-26.
 Roots, Tops and Plant number given.
- Tea. 110. 144 plots, each $\frac{1}{2}$ a. T.A. 2 a. G.
 Eden, T. Studies in the yield of tea. *J. Agric. Sci.*, 21, 547-73.
 Yields and dry matter at 94° C. given.
111. 24 plots. G.
 Vaidyanathan, M. The method of covariance applicable to the utilization of the previous crop records for judging the improved precision of experiments. *Ind. J. Agric. Sci.*, 4, 327-42.
- Timothy hay. 112. 240 plots, each $16\frac{1}{2}' \times 16\frac{1}{2}' = \frac{1}{16}\frac{1}{16}$ a. T.A. $1\frac{1}{2}$ a. G.
 Holtsmark, G. U., and Larsen, B. R. Über die Fehler, welche bei Feldversuchen durch die Ungleichartigkeit des Bodens bedingt werden. *Landw. der Versuchstationen*, 65, 1-22. See also Roemer, Th., *der Feldversuch*. 1st Ed.
113. 35 plots, each $13\cdot2' \times 66' = \frac{1}{8}\frac{1}{16}$ a. T.A. $\frac{3}{4}$ a. G.
 Each year 1929-32 on different parts of the same field.
 Summerby, R. The value of preliminary uniformity trials in increasing the precision of field experiments.
- Tomatoes. 114. 180 plots, each of 6 plants. G.
 Strickland, A. G. Error in horticultural experiments. *J. Dept. Agric. Victoria*, 32, 408-16.

- Walnuts.** 115. 320 individual seedling tree yields. G.
Batchelor, L. D., and Reed, H. S. Relation of the variability of yields of fruit trees to the accuracy of field trials. *J. Agric. Res.*, 12, 245-83.
- Wheat.** 116. 390 plots, each $4' \times 4' = \frac{1}{3000}$ a. T.A. $\frac{1}{8}$ a. G.
Bose, R. D. Some soil heterogeneity trials at Pusa and the size and shape of experimental plots. *Ind. J. Agric. Sci.*, 5, 545.
117. (1) 288 plots, each $8'' \times 7\frac{1}{2}' = \frac{1}{3000}$ a. T.A. $\frac{1}{30}$ a. G.
(2) 288 plots, each $8'' \times 8' = \frac{1}{3000}$ a. T.A. $\frac{1}{25}$ a. R.
Christidis, B. G. The importance of the shape of plots in field experimentation. *J. Agric. Sci.*, 21, 14-37.
118. 3100 plots, each $8'' \times 5' = \frac{1}{3000}$ a. T.A. $\frac{1}{4}$ a. N.
Day, J. W. The relation of size, shape and number of replications of plots to probable error in field experimentation. *J. Amer. Soc. Agron.*, 12, 100-5.
119. 160 plots, each $13.2' \times 19.8' = \frac{1}{100}$ a. T.A. 1 a. G.
Forster, H. C., and Vasey, A. J. Experimental error of field trials in Australia. *Victoria J. Dept. Agric.*, 27, 385-95.
120. 450 plots, each $21' \times 68' (3\frac{1}{2}' \text{ discard all round}) = \frac{1}{81}$ a. T.A. 9 a. G.
Garber, R. J., McIlvaine, T. C., and Hoover, M. M. A method of laying out experimental plots. *J. Amer. Soc. Agron.*, 23, 286-98.
121. 295 plots, each $21' \times 68' (\text{a border of } 3\frac{1}{2}' \text{ all round rejected}) = \frac{1}{81}$ a. T.A. $5\frac{1}{4}$ a. G.
Garber, R. J., McIlvaine, T. C., and Hoover, M. M. A study in soil heterogeneity in experimental plots. *J. Agric. Res.*, 33, 255-68.
Yields obtained by sampling 5 rod rows.
122. 30 plots, artificially constructed in frames each $4\frac{1}{2}' \times 9\frac{1}{2}'$. G.
Garber, R. J., and Pierre, W. H. Variation of yields obtained in small artificially constructed field plots. *J. Amer. Soc. Agron.*, 25, 98-105.

123. 1280 plots, each $\frac{1}{2}' \times 1.6' = \frac{1}{8000}$ a. T.A. $\frac{1}{40}$ a. G.
Kalamkar, R. J. A study in sampling technique with wheat. *J. Agric. Sci.*, **22**, 783-96.
124. 500 plots, each 11 rows $\times 10.82' = \frac{1}{800}$ a. T.A. 1 a. G.
Mercer, W. B., and Hall, A. D. The experimental error of field trials. *J. Agric. Sci.*, **4**, 107-32.
125. 36 plots, each $\frac{1}{20}$ a. T.A. $1\frac{1}{2}$ a. R.
Metzger, W. H. The relation of varying rainfall to soil heterogeneity as measured by crop production. *J. Amer. Soc. Agron.*, **27**, 274-78.
126. 224 plots, each $5\frac{1}{2}' \times 5\frac{1}{2}' = \frac{1}{144}$ a. T.A. $\frac{1}{4}$ a. G.
Montgomery, E. G. Experiments on wheat breeding. *U.S. Dept. Bulletin Bureau of plant Industry Bull.* 269.
127. 63 plots, each $15' \times 112\frac{1}{2}' = \frac{1}{24}$ a. T.A. $2\frac{1}{2}$ a. G.
Morgan, J. O. Some experiments to determine the uniformity of certain plots for field tests. *Proc. Amer. Soc. Agron.*, **1**, 58-67.
128. (1) Winter wheat. 240 plots, each $\frac{1}{1210}$ a. T.A. $\frac{1}{4}$ a. G.
(2) Summer wheat. 230 plots, each $\frac{1}{1210}$ a. T.A. $\frac{1}{4}$ a.
Roemer, Th. Der Feldversuch. *Arbeiten der deutschen Landw. Gesellschaft*, **302**.
129. 48 plots, each $\frac{1}{10}$ a. T.A. 5 a. G.
Roth. Exp. Sta. Report, 1925-26.
130. 1080 plots, each $6'' \times 1' = \frac{1}{8000}$ a. T.A. $\frac{1}{40}$ a. R.
Smith, H. F. Unpublished data.
131. 360 plots, each 9 rows $\times 1$ chain $= \frac{1}{120}$ a. T.A. 3 a. E.
Waite Institute (Adelaide) Report, 1925-32.
Yields filed at the Waite Institute.
132. 1500 plots, each 1 row $\times 15' = \frac{1}{3600}$ a. T.A. $\frac{1}{2}$ a. G.
Wiebe, G. A. Variation and correlation in grain among 1500 wheat nursery plots. *J. Agric. Res.*, **50**, 331-57.

133. 94 plots, each $\frac{1}{100}$ a. T.A. 1 a. N.

Wiener, W. T. G., and Broadfoot, R. The amount of variability which may be expected to occur in a determination of comparative yields in small grains. *Proc. Fifth Ann. Meetings Western Canadian Soc. Agr.*, 17-24.

134. 124 plots, each $33' \times 132' = \frac{1}{6}$ a. T.A. $12\frac{1}{2}$ a. G.

Wyatt, F. A. Variation in plot yields due to soil heterogeneity. *Sci. Agric.*, 7, 248-56.

LAND DRAINAGE: THE AREA OF BENEFIT

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PRIOR to the Land Drainage Act of 1930, drainage legislation was of a piecemeal character. There were numerous authorities, with varied powers and duties, which had, however, one feature in common—no responsibility for the water directly it had passed the boundary of their limited area.

Following the passing of the Land Drainage Act, 1918, an attempt was made to set up more comprehensive authorities, and, up to 1927, 55 new drainage districts, covering an area of 900,555 acres, had been constituted. Of this total, one authority (the Great Ouse) accounted for 484,963 acres. All these districts were based on the assumption that lands up to 8 ft. above the highest recorded flood level were capable of receiving benefit from drainage works, except in the case of lands liable to tidal inundation, where 5 ft. above tide level was adopted as the boundary. This assumption was argued by Counsel and technical witnesses before committees of both Houses of Parliament during the passage through Parliament of the Land Drainage (Ouse) Provisional Order Confirmation Act, 1920, under which the Ouse Drainage District of that year was constituted.

The Report of the Royal Commission on Land Drainage, presided over by Lord Bledisloe in 1927, showed that under the conditions then existing, the interests of neighbouring authorities were in conflict, whereas they obviously ought to be in common. As a result the Land Drainage Act of 1930 became law, and watercourses of all kinds from field to sea have been brought under jurisdiction.

One very important provision of the Act is that Catchment Boards are required to bring within the jurisdiction of Internal Drainage Boards only those areas which, to quote the Act, "derive benefit or avoid danger" as a result of drainage operations. Some working rule for defining such areas had to be adopted, and the pre-1930 practice of including all land up to the contour line drawn 8 ft. higher than the level of the highest recorded flood (except in tidal areas) was

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continued. At a later date, however, the Ministry, in the light of experience, decided that certain adjustments in this policy were desirable, with the result that, in built-up areas, the flood level itself was adopted as the limit of benefit.

The adoption since 1918 of the 8 ft. line for agricultural land has increased the area on which rates can be levied, thus bringing within drainage districts lands that were totally or partially outside the areas of the older drainage authorities. The objection of the occupiers to inclusion is understandable, especially in instances where the existing drainage works are adequate and no fresh constructions are contemplated. Their response to the explanation that the land is henceforward to bear its fair share of benefits hitherto received free, is the very natural one of denying that it benefits in any way: in other words they suggest that a lower contour than 8 ft., or even flood level itself, should be taken.

The Royal Commission, in considering the evidence which they had received on the subject of the area of benefit, contented themselves with the following statement:—

“ . . . the Ministry of Agriculture and Fisheries, in setting up Drainage Boards under the Land Drainage Act, 1918, had to consider what boundary line they could adopt, having regard to the principle of ‘no benefit, no rate.’ We are informed that the Ministry took the best agricultural and engineering advice which was available and decided to bring within the area of rating for drainage purposes all land up to 8ft. above the highest known flood.”

The adoption by the Ministry, after 1918, of the 8 ft. above the highest known flood, was undoubtedly somewhat of a novelty because, prior to that date, the practice had been to confine drainage districts to flood level, or from 1875 onwards, as in the case of the Thames Valley, to go up to 5 ft. above flood level, although there are numerous instances here and there where land is now liable to be rated for drainage rates under some old enactments, which is far above any of these levels.

The purpose of this article is to show that the 8 ft. line is a fair and reasonable limit to adopt for agricultural land. The argument must necessarily be couched in general terms; but it will show that, in average conditions, land up to the 8 ft. contour benefits from adequate drainage. It is not to be expected that the 8 ft. line is a rigid one clearly marking the upper limit of benefit; the complexity of soil types and distribution, and variations in topography, are such, that, for practical purposes, an average value must be assumed. The

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Act of 1930 makes ample provision for meeting special cases, either by exclusion or by differential rating.

The first step in the argument is to consider what happens to the rain. Part may run off, on or through the surface soil, to ponds or ditches: the remainder enters the soil. Of this, some will be retained by the soil and the rest will percolate downwards and find its way by natural drainage (or constructed drains) either to a stream, or to the underground-water-table, which may be close below the soil surface, or hundreds of feet below. In this country, with its adequate and well-distributed rainfall, the annual precipitation exceeds what the soil can hold. The surplus must be drained away from the region inhabited by plant roots, either by natural or artificial means, or both, if the soil is to be maintained in the best condition for agricultural or pastoral purposes. The records of the Rothamsted drain gauges show that, for bare loam soil under an average annual rainfall of about 30 in., approximately 50 per cent. of the rain percolates below the 5 ft. depth. Obviously, the percolation through soil carrying a crop will be less, but there is still a surplus which must be drained away, which is estimated to be equivalent to about 10 in. of rain.

The water that plant roots utilize is that held by the soil. The particles in the upper layers of a properly-managed soil form loose aggregates or crumbs—described by the farmer as good tilth—which behave towards water much as a sponge. They have a high water-holding capacity and can be regarded as innumerable little water reservoirs for the use of plant roots. In the lower depths of soil, where the crumb structure is largely absent, the water is held mainly as little rings surrounding the points of contact of adjacent soil grains, and in the pore-spaces between the grains; these pore-spaces will in general be only partially full of water when drainage has ceased.

The moisture content of the soil is depleted by evaporation into the air and by the requirements of vegetation. When rain falls on the surface, each successive layer of soil aggregates refills its interstices from the new supply: the surplus passes on downwards and continually suffers diminution as each successive layer levies toll on it. If the rainfall is slight, the whole of it may be absorbed by the soil—a not uncommon occurrence in summer—but the usual condition, over the greater part of the year, is for more rain to be

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available than can be held in the upper and lower depths of soil. The surplus constitutes the drainage or percolation water, and there are two reasons that make it desirable for the percolation to be evacuated as rapidly as possible.

First, the aeration of the soil is effected through the pore-spaces: through them, the carbon-dioxide gas produced by the growth activities of roots and micro-organisms escapes into the atmosphere and is replaced by oxygen. If the pore-spaces are choked with surplus water, the gaseous inter-diffusion of oxygen and carbon-dioxide is diminished and plant growth may be adversely affected; crops in this "drowned" condition are suffocating for want of air.

Secondly, in conditions of impeded drainage, the crumbs disintegrate, the soil falls into bad tilth and becomes more impervious to the passage of both air and water. Soil in this state is difficult to cultivate and does not provide the best conditions for plant growth.

Although there is an important change in perspective, there is nothing new in the above conception of the soil as a kind of porous framework, retaining within its interstices (especially those permeating the crumbs) a certain proportion of the rain and permitting the surplus to escape downwards as percolation. But there is still some misconception about the way in which the plant roots obtain their moisture. Absorption of water by the root reduces the soil moisture content in the immediate neighbourhood, and water tends to move into the depleted zone from the surrounding regions of higher moisture content. Although no experimental proof was ever given it was assumed that such movement extended over considerable distances. It is now known that not only is the distance very limited but also that the rate of movement is very slow. The truer picture is that the soil moisture remains relatively stationary, while the plant's ramifying root system traverses the soil so thoroughly that no part of the water is far removed from an absorbing region.

Recent studies show that the root systems of common agricultural plants extend deeper than is usually supposed. The depth varies with the variety of plant, the type of soil and the meteorological conditions, but the following figures are typical: wheat, oats, and sugar-beet, 5-6 ft.; barley, 3-4 ft.; potatoes, 3 ft. Hence, when the water-table is sufficiently below the surface for a 5-foot depth of freely-draining soil to be available for the roots to traverse, this depth of soil can be

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pictured as containing innumerable small reservoirs of water, each being within a short distance of an absorbing root-hair. A 5-foot depth of soil holds water equivalent to $7\frac{1}{2}$ in. of rain at least.

In periods of drought, the soil-moisture is progressively depleted, and the question arises whether at these times the soil can draw on the underground-water-table. Because of the above-mentioned misconception of the distance and speed of water movement in soil, there still exists a belief that water will rise by capillarity to a considerable height above the underground-water-table. This is not so. Experiment shows that, even in conditions of continuous drought, the maximum height of capillary rise is only 3-4 ft. in a heavy loam soil, while for lighter soils the height is, of course, less. Therefore, assuming the maximum capillary rise to be 3-4 ft., and taking 5-6 ft. as the depth of soil inhabited by roots of agricultural plants, it follows that a water-table more than 8-10 ft. (the sum of these two quantities) below the soil surface is, for practical purposes, incapable of supplying water by capillary rise to the roots.

Consider, now, the conditions in a valley. Near the stream the underground-water-level will approximately coincide with stream-level. Proceeding away from the stream, the land level will rise and so will the underground-water-level, but at a lesser angle; hence, the farther one proceeds up the side of the valley the greater is the depth of the underground-water-level below the surface.

In fields near the stream there is only a shallow depth of drained soil for plant roots to inhabit. In spite of this, they will not suffer from drought because the underground water is close below; but a temporary rise in the level may check root activity because the shallow depth of soil will rapidly become nearly full of capillary water, thus reducing aeration and also causing deterioration in the soil structure. This effect will be progressively reduced the farther one proceeds up the side of the valley until, where the underground-water-level is 8-10 ft. below the surface, the plant roots will have the full 5-6 ft. zone for development before reaching the top of the 3-4 ft. zone of capillary rise. It follows, therefore, that everywhere from this point down to the stream, plants are liable to suffer if the underground-water-level is not kept as low as possible, and therefore, lands whose underground-water-level is 8 ft. or less below the surface are

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benefited by drainage works that provide for the rapid and orderly evacuation of surplus water.

It would be impracticable to trace the 8 ft. underground-water contour by a series of borings; some simpler and less costly procedure must be used for administrative purposes. The only practical alternative is to strike a contour on the soil surface a given vertical distance above stream-level, and it is clear that the vertical distance employed could legitimately exceed 8 ft., since the underground-water-level is itself inclined upward from the stream. Nevertheless, the 8 ft. contour is employed in practice, thus excluding from the rateable area a marginal fringe of land that could quite fairly be brought in.

The practice of measuring the 8 ft. contour, not from the stream-level but from the points reached by the highest recorded flood, is also quite justifiable. A flood "banks up" the drainage water that is flowing along the underground-water-contour to the stream and thus progressively raises the underground-water-level in the higher unflooded land. Even though the visible flood may subside quickly, the land it covered will remain saturated for some longer time and so the banked-up water in the higher land will only slowly subside. In addition, there may be deterioration in the tilth of the land that has been flooded, so that it drains less freely than before, and the increased resistance to water movement still further reduces the rate at which the banked-up underground-water in the higher lands can subside.

The direct effects of a flood on the land it covered are clearly visible; but its indirect effects on the higher unflooded land are at least as important.

COMPRESSIBILITY CURVES AS A QUANTITATIVE MEASURE OF SOIL TILTH

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(With Plates VIII and IX and Four Text-Figures)

INTRODUCTION

THERE is at present no quantitative measure of soil tilth. If the farmer is asked to express an opinion on the tilth of a field, he generally presses the soil with his foot and notes the compressibility, the ease with which the lumps of soil disintegrate, the stickiness, and possibly the tendency to recoil elastically when his weight has been removed from the soil. These properties are judged at a compressive stress considerably less than that obtaining in normal cultivation processes. Thus a man weighing 150 lb. if he puts the whole of his weight on his one foot, assuming the area of contact to be 35 cm.²,¹ will exert a stress on the soil of less than 200 g./cm.² whereas, in practice, he seldom shifts more than half his weight on to the foot with which he is testing, so that a stress of the order of only 100 g./cm.² is probably applied. Ballu (1) calculates that an ordinary farm horse exerts, under its own weight, compressive stresses of the order of 2000–4000 g./cm.² if the ground is hard enough for the whole weight to be taken by the shoes, that tractor tyres produce a stress of 1000–3000 g./cm.², and caterpillar wheels of the order of 250 g./cm.², though these stresses are only exerted for very short periods of time. The stresses produced on the mouldboard of the plough are very variable. Nichols (2) has worked over a stress range from about 350 to 2000 g./cm.², and points out that the lower part of this range, although more complex than the upper part, is, “from a practical point of view . . . quite important, as pressures are far above the average pressure exerted by the plow”. Nichols worked on soil carefully prepared in “a fluffy finely divided state, without the formation of lumps or puddled particles”.² Work by other authors has generally been confined to soil removed from its natural environment.

In designing an apparatus to measure quantitatively and imper-

¹ Estimated by observing the “wear” on a pair of old rubber boots.

² For a complete account of the development of cultivation processes see Keen (3).

sonally what the farmer gauges by his skill and experience, it is advisable that the weight to be applied to the soil should be considerably larger in area than any soil lumps likely to be encountered. If stresses of the order of 1000 g./cm.² are to be applied, this would involve the transportation and manipulation of many hundredweights of metal, and in practice a compromise must be reached, both stresses and areas being smaller than those theoretically most desirable.

THE FIELD APPARATUS

For this purpose the apparatus shown in Pl. VIII was constructed. The weight consists of four cylinders of iron *A*, diameter 15 in., laid one above the other, each weighing about $\frac{1}{2}$ cwt., the whole weight being hung from a point just above its centre of gravity, so that unevennesses in the soil surface only produce very small restoring forces. This weight is hung from a spring balance *B*, which can be raised or lowered by a windlass *C* operating through a worm, the whole being supported by a tripod fastened by iron pins to a triangle of iron resting on the soil surface. At each corner of this triangle is brazed a circular disk, 8 in. in diameter, to prevent the base from sinking into the soil. This apparatus can be placed in position without any disturbance of the soil beneath its centre, over which the weight initially hangs. The weight is lowered until its surface just touches the topmost summits of the lumps of the soil surface. A duralumin rod *D* is pivoted to the suspension between the spring balance and the weight, and is suspended on a hardened steel knife-edge attached to an independent iron rod *E* bent at right angles at both ends so as to penetrate the soil, and likewise fitted with 8-in. disks. The further end of the duralumin rod is ground to a point, which is trained on to a vertical millimetre scale held by another independently "disked" iron stand *F*.

The weight (approximately 230 lb.) is lowered on to the soil by stages; the effective load on the soil is thus 230 lb. minus the spring-balance reading. The increments of load are applied at $\frac{1}{4}$ -min. intervals, and, immediately before each increment, the reading, *L*, of the spring balance and σ , that on the deformation scale, are recorded. The increments of load are made as nearly equal as possible. Two operators are required for the tests. The principal operator calls the $\frac{1}{4}$ -min. intervals from a stop watch, reads the σ -scale, and records all the data, while an assistant gives a turn to the windlass when instructed, and calls out the spring-balance readings.

Interpretation of the significance of the data obtained will be largely reserved for a later section, but it will be advantageous at this stage to

examine a single experimental curve, such as that shown in Fig. 1. As the load is increased, the deformation increases at first fairly rapidly, and

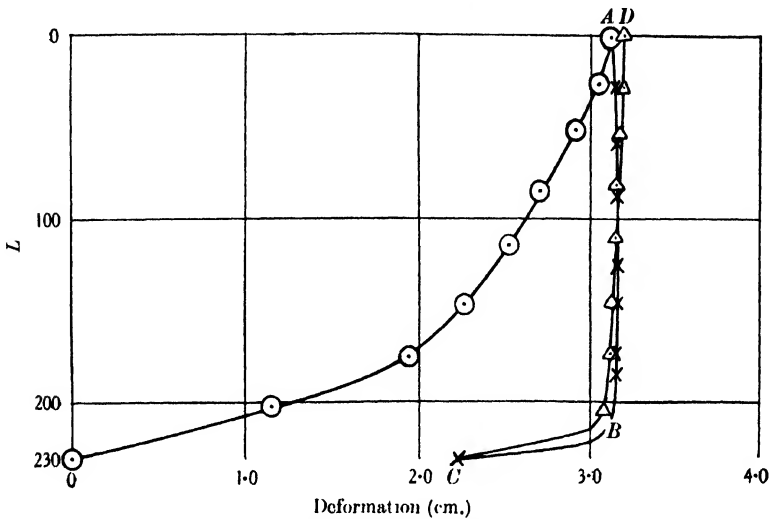


Fig. 1. Load-deformation curve for a field soil.

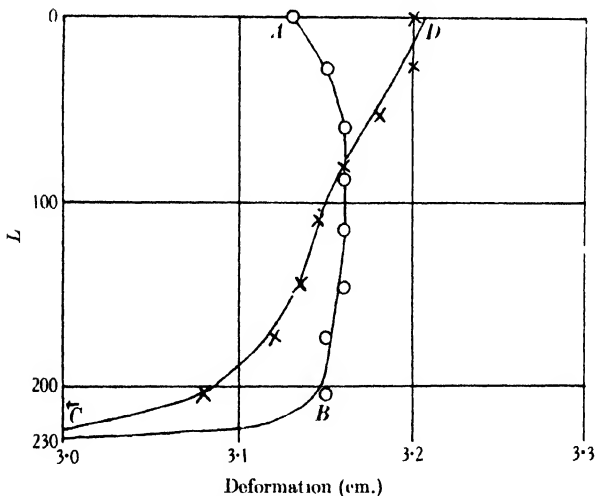


Fig. 1 a. Replotting of data given in Fig. 1 to show elasticity effects.

later more slowly. By the time the whole load is resting on the soil, a total compression *A* has been produced (circles on Fig. 1). This is partly elastic (recoverable) and partly plastic (non-recoverable) (*vide* Schofield &

Blair (4); Nádai (5)). If the load is removed from the soil by stages at the same rate as it was applied (crosses in Fig. 1), the soil surface actually rises very slightly. That this effect is real has been checked by doing tests on hard incompressible surfaces. These tests show that any elastic "give" in the apparatus itself must be exceedingly small. The loading curve, *OA*, is complex in shape, and its form will be considered later. It is by no means invariably of the form shown in the figure, but in all soils investigated the permanent plastic deformation is large compared with the recoverable elastic deformation shown in the unloading arm of the curve *AC*. The tailing off of the curve from *B* to *C* is apparent rather than real, and depends simply on the unevenness of the soil surface and consequent difficulty in assessing a correct zero. In Fig. 1*a*, the scale has been increased so as to magnify the hysteresis loop. The amount of flow which takes place during the unloading and subsequent second loading of the soil (triangles in Fig. 1, crosses in 1*a*) is largely determined by the moisture content, whereas the steepness and shape of the first loading curve depend more on the looseness of tilth of the soil. These latter factors may be subdivided into (*a*) flow and rupture properties of individual soil lumps or crumbs, and (*b*) capacity of these lumps to alter their packing under load. Although the elastic properties of the soil in tilth are interesting, the recoverable deformations are so small (of the order of a millimetre in the experiments shown) that their practical interest is not so immediate as that of the plastic deformations. Nichols (2) has pointed out the difficulties involved in fitting any equation to the part of the compression curve when stresses are relatively low, and before attempting any complete treatment to actual experimental results it seemed advisable to evolve some method of plotting the data which should give a straight line as the ideal case, divergencies from linearity then being treated as a measure of abnormality of one sort or another.

THE "IDEAL" RELATIONSHIP BETWEEN STRESS AND DEFORMATION IN COMPRESSION OF A CRUMB-STRUCTURED MATERIAL

This problem has been touched on by Terzaghi (6) and treated much more completely by Pokrowski & Bulytschew (7). These latter authors point out that in compression, the soil particles become increasingly disturbed out of their original structural formations and suggest an equation in which the stress gradient $dS/d\sigma$ is proportional to the stress at any point on the loading curve, multiplied by the difference between this stress and the limiting stress at which the disturbance of structure is

complete. For small stresses, this equation reduces to $dS/d\sigma \propto S$, in which limiting case straight lines should be obtained by plotting $\log S$ against σ . This treatment has been applied to some of the data from an experiment described in a later section, and the curves, which show a fair linearity, except in cases where the soil is very incompressible⁽¹⁾, are given in Fig. 2. The numbers refer to the treatments described later.

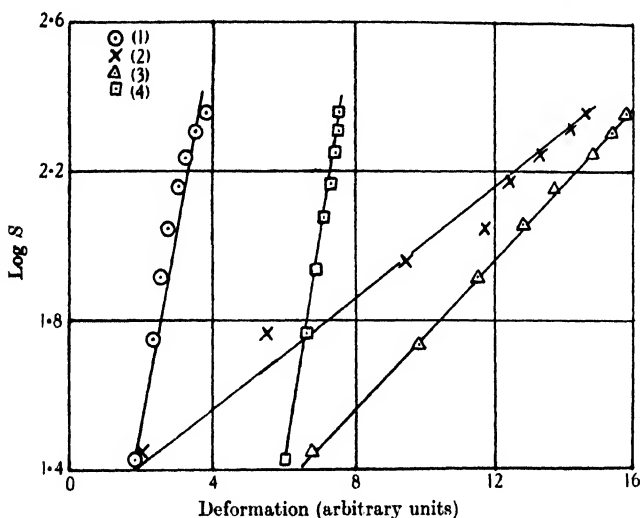


Fig. 2. Data from field soil compression tests plotted according to simplified Pokrowski equation.

It would seem more logical, however, to relate the stress gradient to deformation rather than to stress. The change in structure on compressing the soil may be regarded as a type of work-hardening and certainly depends more on the amount of compression than on the stress, and the total load exerted on the weight by the soil will rise proportionally to the area of contact as the area increases with the sinking of the weight. At first, the area of contact will increase rapidly, whereas repacking and shear effects will be slight. As compression proceeds a region will be reached in which the two processes will have about an equal importance, and here the stress might be supposed to vary ideally with the square of the deformation—varying directly with the deformation for each of the two factors, increased surface of contact and packing and shearing.¹ This is equivalent to assuming that $dS/d\sigma \propto \sigma$ for a crumb-structured material.

¹ The shearing properties of the soil are being very thoroughly investigated by Pigulevski and his colleagues⁽²⁾.

For an elastic solid¹ $dS/d\sigma = \text{constant}$, or S increases proportionally to σ , and an approximation to this condition occurs for very hard dry soils, or at the top of the stress-strain curve, when compacting is considerable. (Experimental evidence for this will be given in a later section.) For a soil the loading curve is thus sigmoid in shape, for, in the lowest stress region, the stress varies as a power of the deformation greater than two, and at the high stress range it varies more nearly as σ . The intermediate region is that in which the repacking, shear, and rupture of the soil crumbs are principally taking place, and this region, where S varies approximately as σ^2 , is in general the more marked the better the tilth of the soil.

It is clear that a very great many data will have to be examined before it can be established as a certainty that this treatment, which is partly empirical, gives the nearest approximation to agreement with the experimental figures. As Nichols rightly says, no true soil will conform exactly to any simple equation. A simple flow equation presupposes that the fine structure of the material is of such an order that statistical laws can be applied. The laws of flow for a true fluid depend on the great number and small size of the shearing units. In the flow of pastes complications arise due to size and shape of the shearing units⁽⁹⁾ and, in natural soil, where these become of the same order of magnitude as the apparatus, individual particles may show their effects on the curves (*vide infra*). Before any information can be obtained as to the individual eccentricities of particular samples, the curves must be reduced to a form where the gross effects of compression have been as far as possible reduced to order. For this reason it seemed good to design an apparatus in which curves could be obtained, giving the deformations plotted against the square root of the stress, the deformation being given at a constant rate. Partly because such an apparatus would be difficult to construct on a field scale, and partly because it was desired to investigate conditions of tilth, some of which could not be conveniently obtained on the farm, the apparatus was made for use in the laboratory. The technique is subject to the criticism applicable to all laboratory tests that the soil is liable to some changes in condition in the process of transferring to the laboratory, however carefully the operation is done. The problems are therefore being studied at the same time by both methods: first, the field soil loading apparatus already described in which loads are applied as far as possible in equal increments after equal intervals of time, the rate of deformation being increased and elastic as well as plastic deformations being considered;

¹ For a true fluid, the stress would be proportional to the *rate* of deformation and independent of the absolute deformation, so that $dS/d\sigma$ would be zero.

and secondly, the laboratory method, in which deformations are given at a carefully controlled constant rate, the square root of the stress built up being automatically plotted against deformation. In this latter method, elastic hysteresis phenomena are not studied.

THE LABORATORY APPARATUS¹

The apparatus is shown in Pl. IX. *A* is an enamelled metal tray (20 × 15 cm.) containing a layer of soil 2.5 cm. deep. (The effect of depth of layer has been investigated and the depth here quoted has been found satisfactory.) This tray is hung by four chains and counterpoised by a bucket *B*, containing water. A constant speed motor *C*, operating through a suitable worm gearing, causes the tray to rise at a constant and very slow rate of about 2.35 mm./min. A lead weight *D* (=1670 g.) is hung from the beam *E* of a counterpoised balance resting on knife edges *F*. The weight is a cylinder of diameter 6.0 cm. It is hung from a point just above its centre of gravity, the suspension passing upward through a wide enough hole to allow for a maximum of about 10° of tilt if the surface of the soil is uneven. The force tending to right the weight is extremely small. Except at the lower end, the suspension is of steel wire to avoid errors due to elasticity in the suspending thread.

As the rising soil surface tends to take up the load of the weight, the beam of the balance rises, thereby opening a valve *G* which allows mercury, stored in the container *H* and kept at a constant head by adjustment of the tap *J*, to run into the bucket *K* which is hung on the same arm of the balance as *D*. This compensates for the change in load produced by the gradual lifting of *D*. The bucket *K* has two of its sides parallel and two sloping, so that the height of the mercury collected is proportional to the square root of its mass, and hence to the square root of the load pressing on to the soil. The bottom of *K* is made flat, and before each test, 2.5 c.c. of mercury are run in from the burette *L* to cover this flat bottom. On this layer of mercury floats a small steel weight attached by means of a cotton passing over pulleys to a pen *M*, the other end of whose holder is again attached to a smaller counterpoise weight *N*. As the mercury lifts the weight in *K*, *N* pulls the pen *M* across the paper which is attached to a glass sheet by two rubber bands. The glass sheet is driven in a direction at right angles to the movement

¹ This apparatus was described, with special reference to its application to soil amelioration problems, at the Conference of the Sixth Commission of the International Society of Soil Science, held at Zürich, August 1937. The author is indebted to Mr D. Morland for much help in the construction of the apparatus.

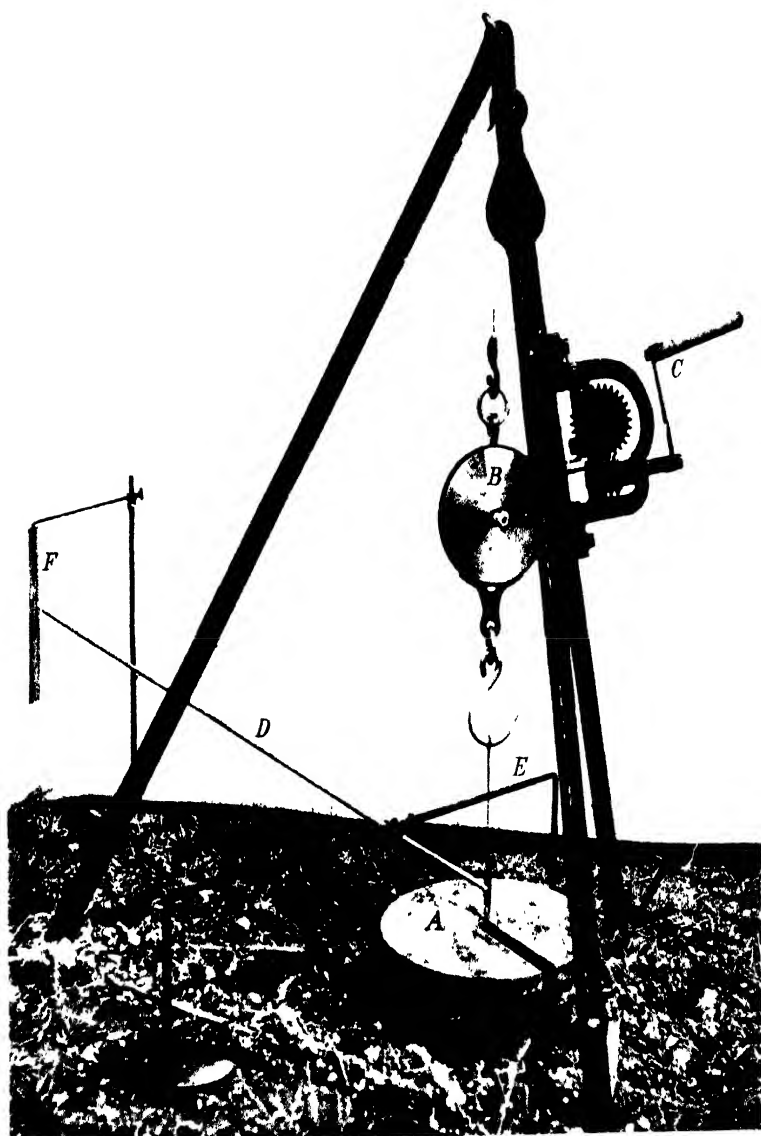
of M by a second gearing from the motor C , and as D does not move appreciably M traces a curve whose ordinate is proportional to the square root of the load on the soil, and whose abscissa is proportional to the amount of deformation. The two axes are drawn, one by raising and lowering N before the test, and the other by the second pen O attached rigidly to the frame, which is aligned so that the two lines so produced are at right angles. The total load represented in the diagrams amounts to 59 g./cm.², though, since only a part of the surface of the weight is in contact with the soil during much of the run, much higher local stresses must be produced. Work with a larger weight has also been carried out, but it is difficult to prevent weights giving high loads per cm.² from becoming unduly top-heavy. Once the motor has been started, it will be observed that the whole process, including the drawing of the curves, is automatic, except only for the adjustment of the tap J , which is a matter of secondary importance.

Data obtained from laboratory apparatus

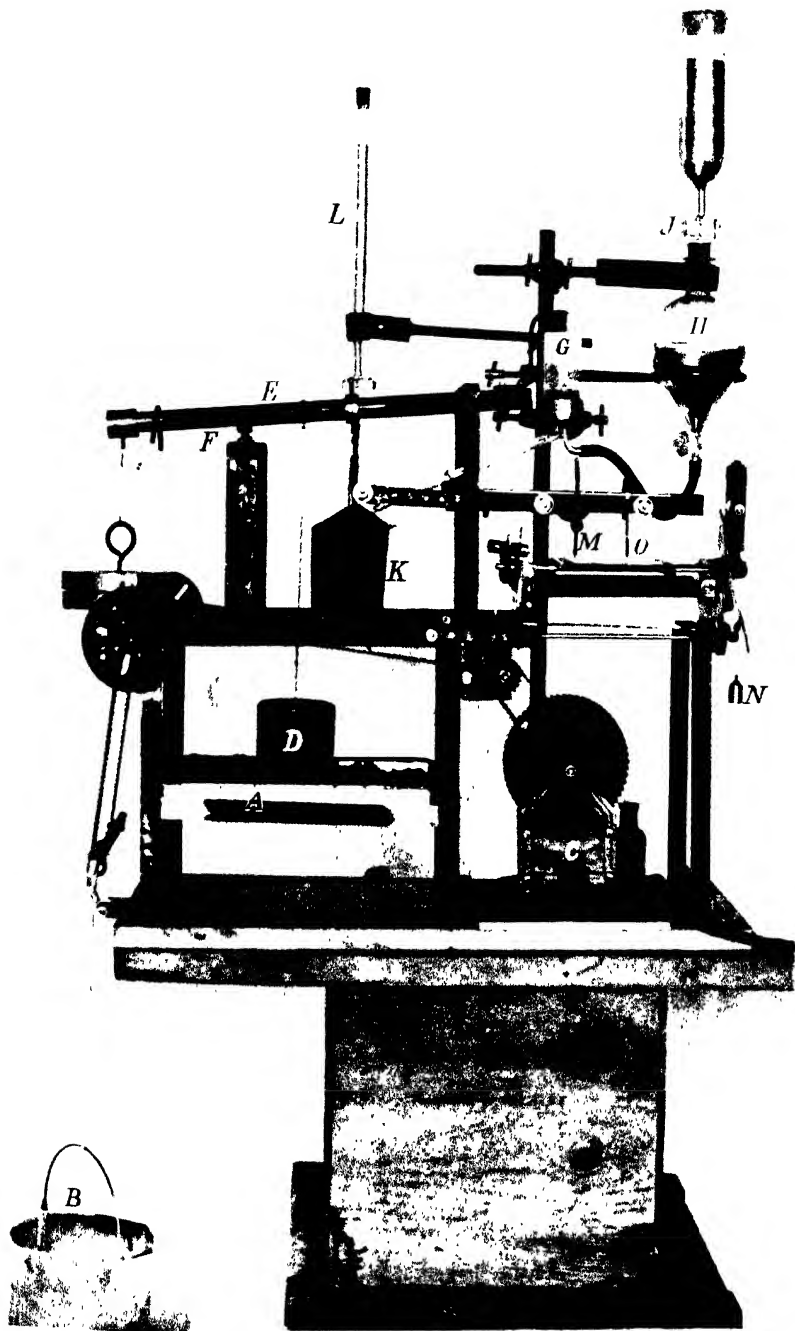
A number of curves obtained with this apparatus are given in Fig. 3: (1) is that for a dry sand, (2) for a wet sand in such a condition that the material coheres into a loose kind of structure, and (3) the same sand wetted to such an extent that the structure again disappeared. It is clear that in the two cases in which there is no crumb structure, the curve is concave to the deformation axis throughout, and calculation shows that the stress varies approximately as the deformation, whereas where there is a structure the curve is predominantly convex, the strain varying with some power of the stress higher than 2. Intermediately, approximately straight-line curves may be obtained. A "step-ladder" formation just visible at the lower end of curve 2 indicates the disintegration of individual crumbs. In Fig. 4*a* values of S calculated from \sqrt{S} readings read off the dry-sand curve are plotted against σ . It is clear that under the circumstances of this test the "elastic" law is approximately obeyed.¹ For comparison, curve 4 (Fig. 3) shows a test made on an ordinary rubber sponge. This is more or less elastic, as is shown from the S/σ curve (Fig. 4*b*). The modulus is not quite constant for low stresses due to peculiar surface properties.

A curve for a wet, structureless soil is shown in Fig. 3, 5, and may be

¹ The laboratory apparatus is not designed to study elastic phenomena, and it is known that there is some "give" in the apparatus itself. For this reason reliable elasticity moduli could not be calculated from these curves.



Field apparatus.



Laboratory apparatus.

compared with that for a soil in fairly good tilth (Fig. 3, 6). The significance of the differences in form will be discussed later.

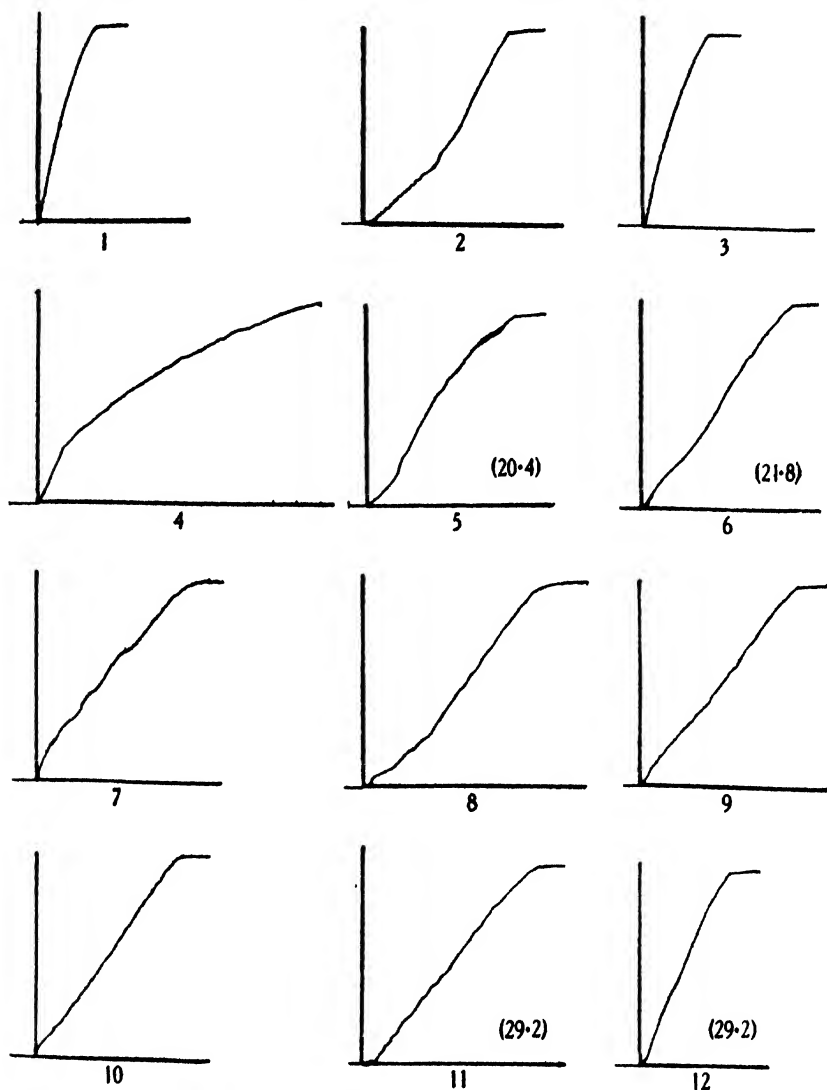


Fig. 3. Compressibility curves from self-recording laboratory apparatus. Ordinate is square root of the load, and abscissa is deformation. Moisture contents are given as numbers in brackets.

It is natural to enquire how far the size of lumps of soil affects the shape of the curve. In order to study this point, the soil used for Fig. 3, 6 was sieved into a series of fractions, curves for the individual fractions

being taken. These are shown in Fig. 3, 7 particles $> \frac{1}{2}$ in., 8, $\frac{1}{2} - \frac{1}{4}$ in., 9, $\frac{1}{4} - \frac{1}{10}$ in., and 10, $< \frac{1}{10}$ in. The differences are surprisingly slight.

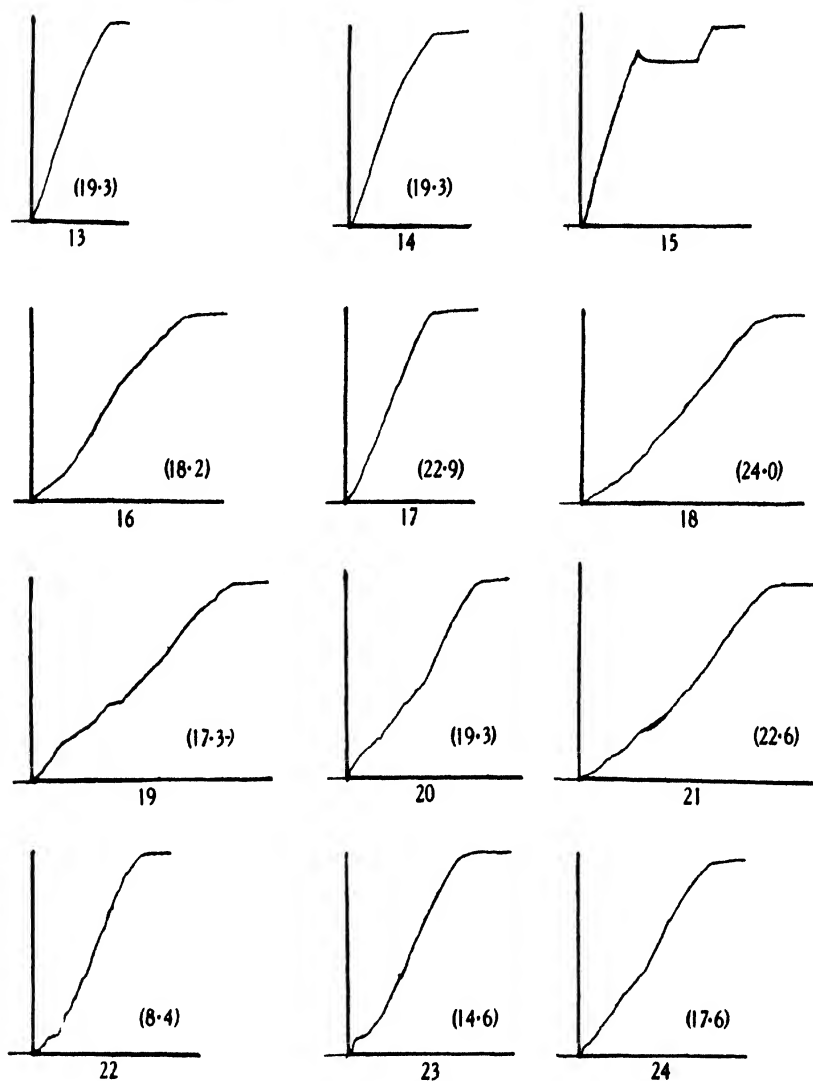


Fig. 3. (Continued).

Except in the case of the smallest fraction, there is a general tendency for the total compressibility to increase with decreasing size of particle, and the biggest particles not only tend to give a somewhat erratic curve, but

the stress clearly varies as some power of the deformation less than 2. This leads to the question as to how far the compression observed is due to shear or crushing of individual lumps, and how far to packing effects. Although local stresses must sometimes be much higher than the mean value of 59 g./cm.², not very many crumbs are actually crushed, except in the case of soils having artificially prepared very soft crumbs. We are mostly concerned with the distribution of deformation between shear and repacking. Mr G. H. Cashen suggested that experiments might be done on the compression of three crumbs chosen as far as possible to be of the

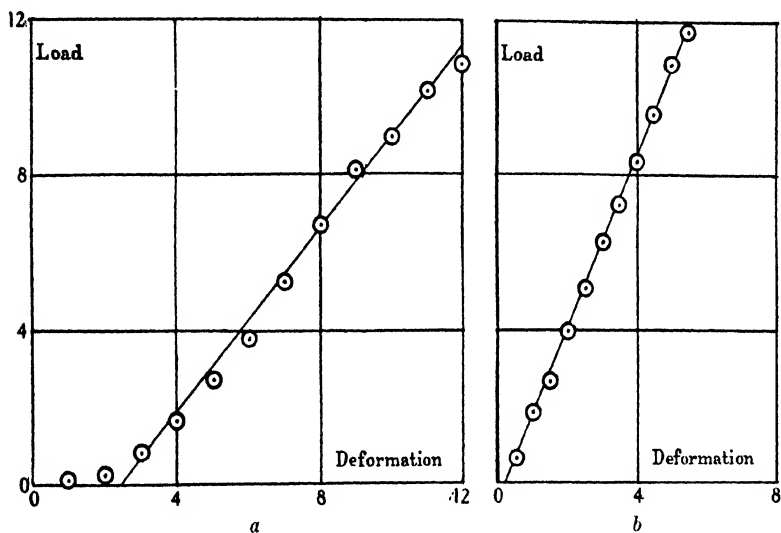


Fig. 4. Compressibility curves plotting deformation against load directly. *a*. Dry sand (calculated from Fig. 3, 1). *b*. Rubber sponge (from Fig. 3, 4).

same size (about 2 cm. diameter), and that comparison should be made with the curves for the complete soil from which the crumbs were selected. The results of these experiments are shown in Fig. 3, 11–15. Fig. 3, 11, is a curve for a surface soil obtained from a wood where the soil condition was kept good by natural processes. The soil lumps were small, and three of the largest of them had to be selected to obtain curve (12). Curve 13 is for a complete soil from the same neighbourhood, but taken from a nearby waterlogged cultivated field which had not been ploughed for some time. The soils had both been somewhat dried out in the laboratory before testing, and the latter soil had set into large hard lumps, three of the smallest of which had to be selected for the test 14. It is clear that the compressibility of the larger lumps from the good soil and that of

the smaller lumps from the bad soil were very similar, though in the former case this compressibility represented only a small fraction of the total compressibility of the soil, whereas in the latter, repacking can have played hardly any part in the building up of the composite curve. In none of these three-crumbs tests did the crumbs crush completely. In order to show the effect of such a collapse on the curve, a soil made into very brittle crumbs in the laboratory was selected, and three crumbs tested. The curve is numbered 15. The apparent fall in stress immediately after the breaking of one of the crumbs (the only complete break during the test) is due to an upsetting of the surface tension conditions round the weight floating on the mercury surface.

EFFECT OF CHANGES OF TILTH ON THE CURVES

If the line of argument followed in this paper has been cogent, it should be possible to follow changes in tilth produced by natural processes by means of the laboratory technique described. Certain effects, such as slight surface "capping", may be incapable of preservation during the process of transferring the soil from the field to the testing tray. If these effects are to be studied the field technique must be used; but many of the changes produced by climatic or cultural processes on the soil-crumbs structure will readily survive transportation. The effect of a spell of frosty weather is shown in Fig. 3, 16-21. (Curves 16-18 are for the samples taken from three locations before the frost: (1) the top of a furrow on ploughed land in fair tilth, though over-wet, (2) a nearby depression where the lack of drainage had produced a really bad condition, (3) an allotment whose soil had been well cared for and suffered only from excessive moisture. (Moisture figures are given in brackets on the figure, and refer to the percentage moisture on a wet basis determined by drying the soil at 110° C. for 24 hours.)

Following a few days of frost, further samples were taken from the same three places. A considerable improvement in tilth is shown in the case of the soil from the top of the furrow, a greater retentive improvement for the waterlogged sample, but little change is found for the soil already in good condition (curves 19, 20 and 21 respectively). A test designed to demonstrate the effect of freezing in the laboratory on a really good garden soil gave a completely negative result, the only changes produced in the curve being explainable by the slight drying out.

Although space considerations preclude the publication of all the curves, the above soils were all tested not only soon after being brought

to the laboratory, but frequently for a period of some days during the drying-out process. After each test the soil was dug carefully to restore the uncompressed condition of the surface and, although the continuous pressing and digging is bound in the long run to affect the course of the drying process, experiments repeated immediately after such treatment agree very closely with the initial tests. It is therefore believed that the results of following such a drying out in this way are of interest. Three curves obtained after considerable drying are shown in Fig. 3, 22-24, which were obtained from the corresponding dried samples used for 19-21. During the same period of time (about 4 days) the better field soil had dried most, the waterlogged soil, on account of its bad structure, had dried least, and the good garden soil intermediately, due, no doubt, to the very large amount of organic matter which it contained. The decrease in total compressibility is clearly marked in all three cases. The shape of the curves has not been greatly affected, except for a slightly increased step-ladder structure in the case of Nos. 22 and 23 where somewhat hard intractable lumps are formed when the soil is dried.

Sticky points and lower plastic limits were determined (Atterberg) on many of these soils, and it was observed that the latter, which is known to correspond reasonably closely to that moisture most suitable for cultivation, also in many cases corresponds approximately to the point at which a well-marked step-ladder formation is observed in the compression curves. Such conclusions must, however, be treated with caution, since the moisture is often not very evenly distributed throughout the soil mass, and a small number of large lumps having a moisture content differing from the mean for the whole sample may affect the fine structure of the curve quite appreciably.

CONCLUSIONS ON INTERPRETATION OF CURVES FOR EVALUATION OF TILTH, AND APPLICATION TO FIELD EXPERIMENTS

The process of compression is not yet sufficiently understood for a full and complete interpretation of the curves in terms of tilth to be possible. In the field experiments, it is not known how the stresses in the soil vary with depth, though preliminary experiments (conducted in co-operation with Mr Cashen) in which closed rubber tubes attached to manometers were buried at different depths in the soil, indicate that although there is probably a time-lag, the compression effect goes down at least as far as the soil has been cultivated. It is intended to extend these experiments and to publish the results in a later paper. In the laboratory technique

the situation is somewhat different. Here, the effect of depth of soil layer is surprisingly slight, and the deformations are partially restricted by the proximity of the sides of the tray.

Even before the laboratory apparatus had been designed, interesting semi-quantitative results had been obtained in the field by testing areas within a small space which had been (1) dug once and rolled, (2) dug once not rolled, (3) dug twice and not rolled, and (4) dug twice and rolled. Tests were done at different spots on these plots chosen in a random manner, and the total compressibilities (arbitrary units), taken from the best straight line on a \sqrt{S}/σ basis to eliminate surface unevenness effects, were as follows:

Treatment	Days after cultivation treatment				
	0	6	11	21	38
1	2.7	4.4	5.5	6.5	4.7
2	14.2 (?)	11.1 (?)	21.5	18.1	15.6
3	11.6	9.0	15.9	16.1	16.3
4	1.9	2.5	3.7	3.7	6.7

(Figures marked (?) were not as accurate as could be desired.)

The first of these experiments (0 days) provides the data used to test the validity of the $(\log S)/\sigma$ equation, and shown in Fig. 2. Only the first loading figures are given. The complete data for No. 3 are those used to show, in Figs. 1 and 1a, the general shape of the curves. A number of other factors as well as total compressibility were considered, and certain regular effects noted, but it seems wiser to confine our attention at this stage to the broadest outlines, since the experiments have not yet been repeated, and it is hoped to undertake further field experiments, which, from the experience already gained, should be of a higher order of accuracy.

It is clear from the above table that, for all treatments, the soils have become more and not less compressible during the first few weeks of digging, an effect probably due to an increase in moist content from 16 to 26 per cent. When the soil has been rolled, this "lifting" effect is very marked. Digging the soil twice in succession has not made it any more but rather less compressible, whether the soil is afterwards rolled or not.

These preliminary results indicate the kind of information which such experiments should give. The following interpretation of the different characteristics of the curves will serve as a working hypothesis, and may be followed with reference to Fig. 3:

(1) Soils in good tilth show a long deformation range in which the \sqrt{S}/σ curves are approximately linear. Upward curvature (increasing

$d(\sqrt{S})/d\sigma$ is preferable to the reverse, and a big total compressibility is generally a sign of good condition.

(2) A long initial range where $d(\sqrt{S})/d\sigma$ is low, especially if the curve is irregular, indicates an uneven surface of rather intractable lumps.

(3) Very wet or very dry soils give curves concave to the deformation axis for most or all of their lengths. The latter usually give big step-ladder effects. A very light powdery soil may give a fairly high compressibility, but the curve is invariably concave.

(4) Some step-ladder effect is advantageous—a perfectly smooth curve indicates a poor structure.

(5) All data are best interpreted in the light of the moisture content of the soil when tested in relation to its Atterberg constants.

These conclusions are derived largely from experience in the laboratory tests. It remains to be seen how far the differences in the method of stress application will cause them to require modification before application to the field data.

SUMMARY

1. A preliminary account is given of experiments on the compressibility of soils in field condition, and two methods for obtaining compressibility curves, one for the field and one for the laboratory are described. The laboratory apparatus automatically draws a curve relating deformation to the square root of the load built up.

2. The theoretical relationship between load and deformation is discussed, the conclusions reached being at this stage semi-quantitative.

3. Laboratory compression curves are shown to indicate the characteristics of soils in various states of tilth, and the effects of drainage condition, frost action, etc. are discussed.

4. Such factors as size of soil crumb, depth of layer tested, and moisture content of soil samples for laboratory studies are considered.

5. Preliminary field experiments are described in which the effect of simple cultivation processes on soil compressibility were measured.

6. Tentative conclusions about the significance of the differences in the shape of the laboratory curves are given, though these may need to be modified, and will certainly be extended following further experimentation.

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SOME ASPECTS OF CULTIVATION AND OTHER POWER OPERATIONS ON THE FARM.

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INTRODUCTION.

This paper deals with two somewhat contrasting aspects of farming that are being investigated by the Soil Physics Department of Rothamsted; both of them concern the agricultural engineer. The first is soil cultivation which is here discussed, not from the aspect of substituting existing horse operations by their mechanical-power equivalents, but from the standpoint, arising from recent research, that many present-day cultivation operations could be reduced or even given up without real detriment. The second is a comparison of electric motors with internal combustion engines for driving barn machinery with the object of comparing the relative power consumption (units of electricity and gallons of fuel) for the same job of work. Comparative figures of this kind are often asked for as a guide by farmers who have both sources of power available.

Although agriculture is the oldest industry, it is the last to become mechanised. Over great tracts of the world long-established methods are still in use. Even in our own country—the home of the machinery that made possible the Industrial Revolution—the horse remains at least as important as steam engines, internal combustion engines, and electric motors, and seems likely to continue so for a long time to come. But in the economic conditions of our age the safest course for the hard-hit farming community is to reduce production costs. While it is true that governments of many countries are endeavouring to improve matters by manipulating selling prices, the methods are tentative and experimental and subject to fluctuations of political opinion. Production costs, on the other hand, are to some extent within the farmers' own control.

At first sight, the utilisation of mechanical and electrical energy in place of human and animal power would seem, as in other industries, the obvious if not the only way of cheapening production. But while this is also perfectly true of agriculture, it is not the whole story; the purpose of this paper is to put the

subject in its agricultural perspective. Agriculture has two great difficulties to face, which are not felt to nearly the same extent in other industries.

First, the farmer's programme of work is largely controlled by weather conditions. Usually his operations can only be arranged one day ahead, and must often be suddenly changed. Improvisations, and sudden peak periods of work, are so frequently necessary that they can almost be regarded as the normal programme.

Second, while the farmer is compelled many months beforehand to arrange his cropping and stock scheme, and to commit himself irrevocably to it, he cannot say what either the quantity or the quality of the produce will be. As a fantastic parallel, where would the motor-car industry be if the factory manager had no means of ascertaining how many cars his machinery and workmen would make, or whether they would be Rolls-Royces or Tin Lizzies? Yet the farmer has no option but to work as best he can under similar difficulties.

Fundamental conditions such as these influence the whole practice of agriculture and account for much that, to the outsider, seems to be mere inefficiency, or unsatisfactory methods, or both. For example, the modern tractor has been designed by engineers primarily to pull cultivation implements, although the power used in cultivation is only a fraction of the yearly power consumption of the average farm. This paradox is resolved when it is realised that although cultivations can fairly be described as an occasional job, they are of vital importance, and it is often difficult to complete them in time, especially in catchy seasons. The tractor, in fact, is of most service to the farmer in peak-load periods that follow unavoidable earlier delays in his programme. For a similar reason the number of farm workers and horses has to be greater than would suffice for the total labour requirements, simply because these are not steady but fluctuating.

In approaching the question of cheapening production costs it is important to realise that more is involved than the substitution of animals by mechanical power; this presents no important difficulties, and agricultural economists with the co-operation of farmers and engineers have produced comparative costings data for horse and mechanical power for farms of varying sizes and types.* To secure the fullest reduction of production costs one must go deeper into the problem and inquire how far the various operations are necessary and justified irrespective of whether they are done by horses or machines.

* See the publications of the Farm Economics Branch, University of Cambridge; the Agricultural Economics Research Institute, Oxford; and the Department of Economics, Wye College, Kent.

THE ART OF CULTIVATION.

Although the various operations involved in cultivating the soil have been raised to the level of a fine art by the British farmer, nevertheless little or no critical experimental work on cultivation has been done, until recently ; compare this situation with the much later development of artificial fertilisers which have been, and still are, the subject of innumerable and careful plot experiments. Cultivation is an art ; it is not yet a science. It is the most expensive single item in the arable farmer's budget : far more costly than artificial manuring, on which so much money for experiments has been spent. Cultivation is an obvious problem to tackle in the search for cheaper production costs. The neglect to do so is a consequence of the understandable feeling of all good farmers that such an essential part of their work demands all the care and skill they can give it. Thus, in the seventies of the last century, when cheap and abundant human and horse labour were available, we find that the spring sowing of the root crop in particular was preceded by several ploughings and innumerable harrowings and rollings. To quote from a paper given by a prominent farmer at one of the Rothamsted Conferences* : " I have here the tillage book made when my father entered my present farm in 1870 ; there were 4 ploughings, 25 drags, 10 small harrows, and 8 rolls to get a tilth for swedes, and this was in a dry season." Nearly 50 cultivations for a crop that to-day does not pay for its growing ! Although economic conditions have long since precluded such lavish attention, it is still true to say that the farmer aims at getting what he considers to be the correct tilth for each crop he sows, and that he believes the crop will suffer by just the extent that the soil tilth falls short of perfection. It is this belief that calls for critical and impartial examination. In other words, how sensitive and responsive are farm crops to the state of the cultivation seed-bed ; what is the effect, if any, of surface cultivations while the crop is growing ; is a perfect tilth essential for a satisfactory crop, or does the farmer get nothing but the satisfaction of artistic achievement from the extra labour ?

DEEP VERSUS SHALLOW CULTIVATION.

The average American farmer is popularly supposed to be concerned far more with the financial than the cultural or artistic aspects of agriculture ; which may explain why investigators in the United States Department of Agriculture† have conducted

* Rothamsted Conference V (1927) " The art and science of cultivation." (page 16).

† Chlcutt, E. C. and Cole, J. S. *J. Agric. Res.*, 1918, *14*, pp. 481-521. Sewell, M. C. *J. Amer. Soc. Agron.*, 1919, *11*, pp. 269-290.

extensive experiments in the Great Plains on the relation between cultivation and crop yields. Their conclusions were somewhat startling, even when allowance is made for their pre-occupation with restricted rainfall or "dry-farming" conditions. Deep and thorough cultivations were unnecessary; shallow ploughing gave as great yields. The purpose of ploughing was to bury weeds and bulky crop residues or manures, to reduce weed competition, to provide a soil surface that could easily be loosened before drilling the seed. The unmistakable inference is that cultivations beyond those necessary to suppress weeds and provide an initial loosening of the soil are of no benefit to the crop.

In this country, and indeed within all the humid temperate zone, the virtues of deep and thorough cultivation are stressed in most text-books of practical agriculture, but until recently no crucial experiments have been made to test this belief. For some years past the subject has been under careful investigation at Rothamsted, and the conclusions are in general accord with those quoted above. The soil is a heavy loam classed as "clay with flints"; and the subsoil is distinctly heavier than the surface.

In the experiments subsoiling has often been tried, with little or no result. Occasionally an increase of half a ton of potatoes was obtained (just about sufficient to pay for the cost of the work) but no beneficial effect was secured in the following crops. Finally, in a plot experiment on sugar beet, the subsoil was hand-dug and broken up with forks, producing a much greater disintegration than an ordinary subsoiler, but in spite of this no increased yield was obtained. In later experiments, still proceeding, comparisons are being made of deep and shallow ploughing both for grain and root crops. The yields show no consistent benefit from deep ploughing even on the plots that are deep-ploughed each season, where some cumulative effect might have been anticipated; in fact, shallow cultivations frequently give slightly increased yields.

Although the above results apply only to the soil and weather conditions at Rothamsted, our land resembles a considerable area of this country. It is at least open to question whether, elsewhere, subsoiling or indeed any form of deep cultivation will necessarily produce increased yields.

SURFACE CULTIVATION.

Turning now to the question of the value of surface cultivations in the growing crop, it is commonly stated that in addition to destroying seedling weeds they directly benefit the crop. Such cultivations are common in the root-break which is the "cleaning-crop" of the rotation, and cultivations in excess of the minimum needed to keep down weeds are often given. The value of such intensive cultivation in comparison with the

minimum needed for weed removal was tested, both for sugar beet and kale, and on the light sandy soil of the Woburn Experimental Station as well as at Rothamsted. The results were both striking and disconcerting. Far from producing a benefit, the extra cultivations actually reduced the yield. This reduction was statistically significant in that it was more than could be attributed to experimental error. Hence, like deep cultivation, the virtues of intensive surface cultivation are not self-evident.

Obviously much further investigation under varied soil and weather conditions is desirable before coming to final conclusions. It is therefore satisfactory to note that the Cambridge University School of Agriculture has already begun, on its own farm, experiments in which certain standard cultivation practices, regarded as essential by the practical farmer, are being critically examined.* So far their experience conforms closely with that of Rothamsted: many of the cherished beliefs of the farmer appear to have no real basis so far as the final yield is concerned. Differences in early growth were often observed—they are a common feature of the Rothamsted experiments also—but the effects disappear before harvest. It is highly probable that many of the tenaciously-held opinions of farmers are based on these early differences coupled with the understandable, but unproved assumption, that such differences must persist throughout the plant's life.

ROTARY CULTIVATION.

An interesting instance of the evanescent nature of early growth differences is afforded by the Rothamsted experiments on rotary cultivation which have been made since 1926. The experiments are designed to test over a series of years the extent to which rotary cultivation can replace the traditional methods of cultivation on medium-land arable farms. (It may be remarked that rotary cultivation is already well established in orchards and market gardens.) Our experiments show almost invariably that germination and early growth are better on the rotary cultivated plots, but the difference does not persist: at harvest no superiority is found. The initial advantage is probably due to the much looser and softer tilth given by the rotary cultivator, as separate measurements of the amount of disintegration produced by each form of cultivation show, contrary to the usual belief, that rotary cultivation produces not a finer but a much looser tilth than the traditional methods. This kind of tilth, and the fact that the revolving tines tend to distribute weed seeds throughout the whole depth of cultivation also probably explain the increased weediness of rotary cultivated plots as

* H. G. Sanders, J. Farmers' Club, 1935, pp. 81-100.

compared with those cultivated by traditional methods, where the germination is confined to the shallow surface layer. But in spite of these practical difficulties of increased weediness and looser tilth, the yields of arable crops are as great with rotary cultivation as with the older methods ; these results strongly suggest there is nothing sacrosanct or inevitable in the careful sequences of ploughing, cross-ploughing, cultivating, harrowing and rolling that the skilled farmer employs to create a tilth. Hoskyns said the same thing much more forcibly, 70 years ago,* and while the present-day salesman's slogan of "a seedbed in one operation" for rotary-cultivator machines may be somewhat optimistic, it is not altogether off the target.

Enough has been said to show that much of what has long been regarded as essential in the art of cultivation must now submit to the onus of proof ; but it must also be added that even if they fail to pass the impartial tests of science, there will still be left more than enough to give full scope to the cultivator's personal judgment. Conditions—especially the weather—vary so much from one season to the next, and even within the season itself, that no series of objective rules, although based on a fully developed science of cultivation, could completely replace the intuitive decision of the skilled practical man.

THE ELECTRIC MOTOR VERSUS INTERNAL COMBUSTION ENGINES.

There are few farms to-day which do not use some form of power either in the form of tractors or stationary oil engines, or both, for the various operations in and around the farm buildings. At the present day the extension of electrification schemes is providing more farms with the opportunity of using electricity instead of internal combustion engines for this work, although it may be assumed that the use of electricity will be confined to farms not too distant from urban areas, owing to the high cost of bringing electricity to truly rural areas.

With regard to field operations on farms, it may be mentioned that electrically operated ploughs have been developed both in this country and on the Continent, but at present there does not appear to be any great future for this application of electricity. It may be concluded that the use of electricity will be confined to operations in or near the farm buildings.

Farm machinery is usually driven by belt drive from counter shafting, when the change to electric drive merely entails the choosing of a suitable pulley-size for the motor, farm machinery being generally designed to run at a comparatively low speed. Provision of an individual drive for each machine is sometimes advocated, as power losses and dangers connected with overhead

* "Talpa, or the Chronicles of a Clay Farm."

shafting are eliminated : direct coupling is possible in some cases and avoids belt losses. However, it must be remembered that while unit drive may be ideal for some industrial purposes where each machine works say eight hours each day regularly, conditions in agriculture are quite different. Here a number of machines may be required for various operations, but any one may be used for only a few hours per week : again, the use of others may be purely seasonal. These considerations, and those of initial outlay, suggest that a farmer will try to keep down the number of his motors to a minimum, and have these general service motors so mounted that they may be fixed in position, but also moved without difficulty should their occasional use be required in another location.

The process, in the main, has been to adapt motors to existing machinery, but the reverse process may well take place, and some machines, if the amount of their use warrants it, may be adapted to the high speeds obtainable with motors. It might be suggested, for example, that a grinding mill running at 1,400 r.p.m. would be more efficient than one running at 600-800 r.p.m. : if so, would the increase in efficiency be great enough to justify such a change in design, or are there any disadvantages serious enough to make such a change undesirable ? Questions such as these, the mechanical engineer will be able to answer.

We at Rothamsted are not directly concerned with such questions, as our work has been simply to compare motors and internal combustion engines as power sources for the machinery we possess, in the hope that the information obtained—from a neutral standpoint—would be of some assistance to farmers interested in the two types of power. Probably most farmers would have a natural suspicion that the figures given by the manufacturers of engines and motors err on the side of optimism. Our primary aim was thus to obtain comparative electricity and fuel consumptions for the same jobs of work and so enable a farmer to find running costs for his own conditions : this is, of course, putting the matter in its simplest form, as the final comparison must take into account items such as the cost of equipment, depreciation and maintenance and, in the case of electricity, any charge for bringing the supply to the farm.

It should be remembered that the definite advantages of electricity, particularly for lighting, cleanliness, simplicity in operation, increased comfort in the farmhouse as well, have a value which is difficult to assess in terms of money. The consideration of such advantages may well outweigh any disadvantage of electricity as regards power costs when compared with internal combustion engines.

The experiments made to date have been on threshing wheat, oats and barley, and on the grinding of barley for meal.

THRESHING.

The Marshall threshing machine used has a drum width of 48 inches and was driven in turn by three sources of power, viz. :—

- (1) A General Electric Company Witton 20 h.p. portable motor ;
- (2) A new International Harvester Company 10-20 tractor ;
- (3) An old International Harvester Company 10-20 tractor, in use at Rothamsted since April, 1928 and still in a fair condition after 7,000 hours' work.

The experimental threshing was arranged in periods of two hours, to provide some replication for the measurements for each form of power, and to limit to some extent the errors which might occur in the comparisons. Such errors can occur owing to variability of the crop in the stack, and to decrease in the height of the stack as threshing proceeds: when the stack is low more work is required to feed the crop to the thresher, and the tendency is for the rate of feed to be low. Electricity consumptions were obtained from the circuit and master meters, and fuel consumptions by the tractors by weighing. Other observations included weights of graded grain, straw, etc., time and labour for preliminary alignment of the power source with the thresher, the petrol required to warm the tractor engines before turning over to paraffin, thresher drum and drive pulley speeds. A summary of the results is given in Table I where any differential effect of the crop has been ignored.

TABLE I.

	Mean electricity or fuel consumption per hour	Thresher drum r.p.m.
20 h.p. motor ...	7.6 kW 8.3 kW 8.0 kW all experiments	1,070 1,200 1,070-1,200
New tractor ...	1.42 gallons	1,050-1,134
Old tractor ...	1.36 gallons 1.40 gallons all tractor experiments	1,060-1,200

Table I shows that for four experiments at the lower drum speed the mean consumption was 7.6 kW, and 8.3 kW for the higher speeds. The general mean for all experiments was 8.0 kW., which at an efficiency of 85 per cent. corresponds roughly to an output of just under 10 h.p.

The average paraffin consumption for the tractors has been 1.4 gallons per hour, but the individual readings showed greater deviation from the mean than do those for the motor. This is understandable in that the motor automatically draws the required power from the main, but with the tractors wastage will occur if the carburettor is not adjusted to give the weakest mixture possible for the work. For this reason the figure of 1.4 gallons is perhaps rather high, and 1.3 would be a more suitable figure. However, taking the averages as they stand, 8.0 kWh's are equivalent to 1.4 gallons of paraffin, or the paraffin equivalent of 10 kWh is 1.75 gallons.

The measurements of the different grades of threshed produce and a close examination of their condition gave no support for the statement sometimes advanced that the smoother torque of the electric motor produces a better sample of threshed grain: nor did there appear to be any marked effect of changes in drum speed within the limits used—1,050-1,200 r.p.m. No trouble was experienced with the motor and new tractor, but on one occasion the fuel feed pipe on the older tractor required attention.

It should be stressed that in these experiments both motor and tractors were working at only 50 per cent. of their full capacity: the efficiency of a motor falls less rapidly with reduced loads than that of an internal combustion engine: hence if smaller units of 10-12 h.p. had been used, which would have worked at nearly full load, it is probable that a lower figure than 1.75 gallons for the paraffin equivalent of 10 kWh would have been given. This raises an important point in farm practice. Even on the most efficiently run farms the tractor spends only a small fraction of the year in cultivating, harvesting and haulage work. While it may be inefficient in the engineering sense of the word, to employ it for driving barn machinery that requires only a fraction of its available horse-power, from the viewpoint of farm practice the matter appears in a different light. The farmer is "wasting" only the difference between the fuel consumption of the tractor and a smaller engine, but against this he saves the cost of the smaller machine. In the case of the motor, if threshing is the heaviest work it will be called upon to do, there is obviously no need to have more power than is required for this operation. It is true that the difference in efficiency between a 20 h.p. and a 10 h.p. unit is small, and will not lead to a large difference in

power consumption, but there are other ways in which the smaller motor will be more economical. If the farmer buys his current on a two-part tariff, for example, £4 per k.v.a. maximum demand over a period of, say, twenty minutes, plus a unit charge, his maximum demand and hence his fixed charge is increased by a poor power-factor. Comparing a 20 h.p. motor at half load, and one of 10 h.p. at full load, the difference in maximum demand would be approximately 1 k.v.a., or £4 a year difference.

CALCULATION OF COSTS (THRESHING).

The purpose of the Rothamsted experiments is to compare equivalent consumptions of fuel and electricity to provide a basis for comparing relative costs for the two forms of power. The electrical installation at Rothamsted is designed for both experimental and normal farm work and is probably more detailed than would occur under commercial conditions: hence our own costings data would not apply directly to the latter. In the following calculations typical farm commercial conditions for a farm of similar size and cereal acreage to Rothamsted are assumed. The motor considered is one of 15 instead of 20 h.p. and it is assumed that it would be used for other farm work besides threshing, thus increasing its annual use from 200 to 500 hours.

STARTING AND RUNNING COSTS.

The only labour costs which have been considered are those for lining up the source of power with the threshing machine. During actual threshing the number of men employed was the same whether the motor or tractor was used, and the labour costs in threshing are therefore the same for all sources of power. Labour is estimated at 1s. per hour for the tractor driver and 8d. per hour for general labour.

The price of the petrol used was 1s. 2½d. per gallon and of the paraffin 6½d. per gallon. Lubricating oil on the basis of one pint for each experiment cost 1·9d. per hour. The electricity tariff was 1d. per unit in the first year of the experiment and ½d. per unit in the second. There is also a fixed quarterly charge, which is designed to cover the overhead charges for the installation and calculated to give the producer a return of 6d. per unit for lighting. Once the overheads have been covered in this way by the lighting load, the electricity concern can supply electricity for power at a price little above the cost of its production. In our costings we have preferred to spread out the fixed quarterly charge over the total number of units used for all purposes in the quarter. Depending on the quarter this has the effect of increasing the unit charge from 0·2 to 0·4d. For

the purposes of this paper the net cost will be taken as 0·84d. per kWh.

OVERHEAD COSTS.

The overhead costs are strictly those for maintenance and depreciation ; interest on capital invested is actually a charge against profit and not a cost, but it has been included to show the effect of different capital investments.

A knowledge of the working life and maintenance costs of each item of equipment is essential but where such information is lacking, as for electrical equipment on farms, some assumptions must be made. A motor under industrial conditions might be expected to have a life of at least 20,000 hours, which would correspond to a life of 40 years at 500 hours' use per annum. The damper conditions on a farm, combined with intermittent use, would result perhaps in a somewhat shorter life. Again, the life and maintenance costs of the circuit are not known.

We have used the following tentative estimates, supplied to us from authoritative sources :—

<i>15 h.p. motor</i>	cost £69 10s. 0d. Life 20 years at 500 hours per annum. Depreciation $7\frac{1}{2}$ per cent. Maintenance 2 per cent.
<i>Circuit.</i>	Cost £20. Life 25 years at 200 hours per annum. Depreciation $7\frac{1}{2}$ per cent. Maintenance 2 per cent.
<i>Tractor</i>	Cost £230. Life 8 years at 1,000 hours per annum. Depreciation $22\frac{1}{2}$ per cent. Maintenance 3·75 per cent.

The depreciation rates are those allowed for income tax purposes, and the interest charge has been taken at 5 per cent. of the value of the plant. The overhead charges calculated are the averages over the working life of the plant.

The costs for lining up the tractor with the thresher is of the order of 3½d., while for the motor where more labour and time is required, the costs were slightly greater, approximately 4½d.

The costs for the actual threshing are given in Table II.

TABLE II.

CHARGES FOR ONE HOUR'S THRESHING.

(15 h.p. motor : 10-20 h.p. tractor. Output : 2 tons of grain per hour).

	Electrical equipment d.	Tractor d.
Fuel + oil : or electricity ...	6·7	11·0
Overheads	4·8	9·4
Total	11·5	20·4

For the conditions assumed, and consumptions found, the total costs would be the same if the electricity for one hour cost 15·6d., i.e. for a net cost of 1·95d. per unit.

BARLEY GRINDING.

Comparisons were made between a 6 h.p. Bamford diesel engine and a 5 h.p. General Electric Company "Drumotor" when used to drive a Bamford 2C combined grist mill. The comparisons are less straightforward than threshing, as the quality of the product has a much greater effect on the power consumption than in threshing. Preliminary work demonstrated that the factors having greatest influence on the power consumption were rate of grinding, fineness of grinding, quality and moisture content of the barley used. The effect of rate and fineness of grinding on power consumption are shown in Fig. 1, which represents for four rates of grinding the energy per ton required to produce a meal containing varying percentages of fine material : fine material is here defined as that which passes through a No. 32 grit gauze sieve (0·605 mm.). The curves show that for a given degree of fineness the greatest efficiency of the motor and mill occurs for the highest feeding rate, and illustrate the wide range of energy requirement per ton of ground meal possible when both fineness and rate of grinding are altered. The fineness index of course can only be obtained by a sieving analysis after the grinding is finished, and therefore, to compare two sources of power it is necessary to have the fineness-energy curves for each at the same feeding rates. In practice it is most convenient to arrange the required rate of feed, and take a series of observations of power requirement for different settings of the mill plates. The "spot tests" last for a minute or so, during which time the output of meal is weighed, a sample

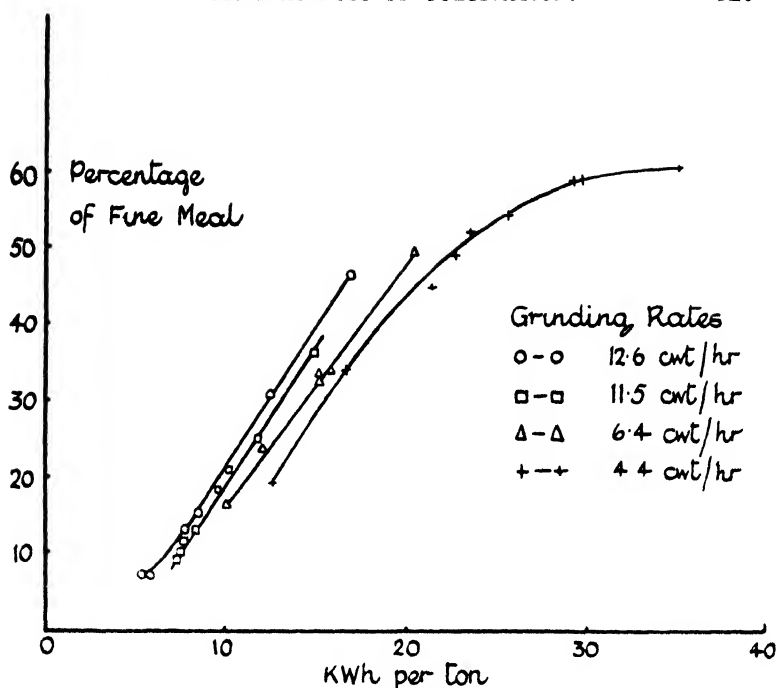


Fig 1 Energy per ton For varying fineness.

is taken for sieving analysis, and the electrical energy or fuel measured. The advantages of this method are that fineness and rate of feed are less likely to change during a short test than in a long one: the amount of grain required is cut down to a minimum, which makes it easier to ensure a uniform quality of grain in the experiments.

The electrical energy is obtained by timing the meter disc with a stop-watch, and the diesel oil consumption by finding the time required for the use of a known volume of oil, usually 25 or 50 c.c.'s. Two sets of curves for feeding rates of 4.0 and 5.6 cwt. per hour are shown in Fig. 2, where output of fine meal per hour is plotted against kilowatts and pints of diesel oil per hour. The measurements with the motor extend over a range of power input of 3.65 to 4.88 kilowatts for the 4.0 cwt. grinding rate and from 3.1 to 5.9 kilowatts for the higher one. The readings with the diesel at the 5.6 cwt. feeding rate starts at a somewhat higher power than that in the corresponding motor experiment: as a result, the agreement of the values of the kilowatt hour-oil equivalent obtained for the two grinding rates can only be checked over a power range of 4.5 to 5.3 horse-power. Within this range the two comparisons agree in giving a consumption of

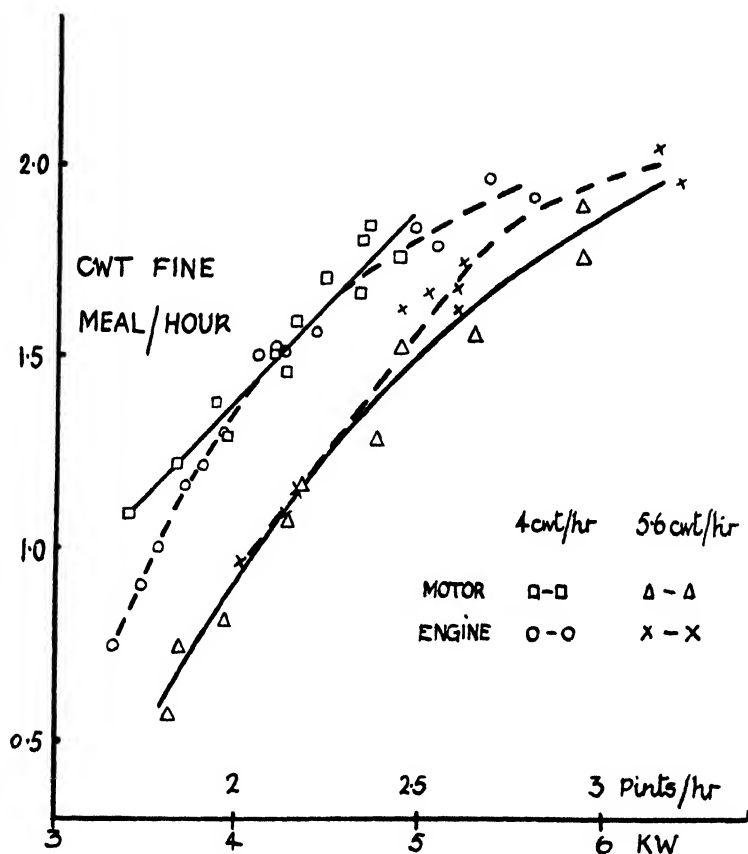


Fig. 2 Power, and OUTPUT of fine meal per hour.

0.46 pints per b.h.p. hour, or 0.49 lb. of fuel per b.h.p. hour, and the diesel oil equivalent of 10 kWh is 5.0 pints of diesel oil. Consumption of lubricating oil by the diesel, according to the manufacturers' recommendations, will amount to one gallon per 200 hours.

The same methods of costing described for the threshing have been followed.

The following estimates have been adopted :—

Diesel engine. Cost £73. Life 10 years.

Depreciation 15 per cent.

Maintenance 5 per cent.

Drumotor. Cost £37 10s. 0d. Life 20 years.

Depreciation 7½ per cent.

Maintenance 2 per cent.

Circuit. Cost £5. Life 25 years.
 Depreciation $7\frac{1}{2}$ per cent.
 Maintenance $2\frac{1}{2}$ per cent.

All the above have been assumed to be in use 500 hours per annum, and electricity has again been taken at 0·84d. per kWh.

The price of the diesel oil we have used depends on the size of the drum in which it is delivered, and ranges from 5½d. per gallon for 500 gallons to 1s. 2½d. for a five-gallon drum. The price for a 40-gallon drum, 8½d., has been taken, as this would be the most usual size for farms. Lubricating oil has been charged at the same price as for the tractors.

The costs for one hour's grinding are given in Table III.

TABLE III.

CHARGES FOR ONE HOUR'S GRINDING.

(6 h.p. diesel : 5 h.p. Drumotor : each at 5 h.p. output.)

	Electrical equipment d	Diesel d.
Fuel + oil : or electricity ...	3·9	2·5
Overheads	1·7	5·5
Total	5·6	8·0

Table III shows that for our conditions, and the assumptions used, the motor is the cheaper unit. Had the net cost of electricity been 1·36d. instead of 0·84d. the costs of the two forms of power would have been the same.

THE SCIENTIFIC BASIS OF THE ART OF CULTIVATION

A paper read at
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at Oxford, January 1937

by

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THE SCIENTIFIC BASIS OF THE ART OF CULTIVATION

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Introduction

THE importance of the physical properties of the soil in soil management has been recognized by agriculturists from time immemorial. When scientific men turned their attention to agriculture, these properties were among the first to be studied. Later the great nineteenth-century advances in agricultural chemistry held the stage, and little further work was done in soil physics. Hence the early treatment survived, although it was rudimentary and defective, and its essentials are still commonly employed in text-books of practical agriculture in connection with the purposes and effects of cultivation operations.

Again, until recent years few field studies have been made on the effects of various cultivations on the growth and yield of crops. In contrast with artificial fertilizers, for example, which have been and still are the subject of innumerable field experiments, cultivation has been totally neglected; and yet the cost of cultivation is far more than that of manuring. The neglect can be attributed to the fact that the art of cultivation was established long before the experimental outlook was developed, and to the natural feeling of the practical man that the operations were so self-evidently fundamental as to be outside any need for experiment.

Nowadays the changed economic conditions of farming no longer permit the almost innumerable cultivations that were standard practice in the 'golden age' of British farming. Who could afford to give to-day 4 ploughings, 25 drags, 10 horse harrowings, and 8 rollings to prepare a seed-bed for swedes, and that in a dry season? ⁽¹⁾ Cheap and unlimited labour has gone, never to return. To-day cultivation costs form the heaviest single item in the arable farmer's budget and, wishing to reduce these costs to the minimum, he is turning to mechanization, which has

effected marked reduction in other industries. But the farmers' problem is deeper than the substitution of mechanical for animal power; the whole art of cultivation has to be examined to ascertain—irrespective of the type of power used—how far the various operations themselves are essential, and whether they can be reduced, telescoped, or replaced by new operations without causing deterioration to the land and the yield and quality of crops. It is precisely here that the relative absence of scientific knowledge about the highly developed but empirical art of cultivation is severely felt.

There are two ways of remedying the deficiency which will ultimately link up with each other: direct field experiments on cultivation such as are now in progress at Rothamsted and Cambridge, and research on the physical properties of soil, on which soil tilth ultimately depends. Of these physical properties, the behaviour of the soil in regard to the moisture which it contains is perhaps the most important. Many traditional cultivating operations have as their main object the control of soil moisture, but until we fully understand how water actually moves and behaves in the soil, we cannot be sure that these operations are really as effective as tradition would have us believe. In this paper modern scientific theories of water movement are examined in the light of their application to cultivating operations in practice.

The early theory of water movement in soil

The early investigators based their theories of water movement in soil on capillary action. As is well known, when one end of a 'capillary' tube—that is, a tube of very narrow bore—is dipped below a water surface, the water will rise in the tube to a level higher than that of the surface into which it is dipped. This action, which is due to surface tension, is such that the narrower the tube, the higher is the level to which the water will rise. Early soil scientists pictured the pore-spaces in soil as consisting of a series of irregular capillary tubes in which water from the natural water-table would tend to rise to the surface. The supposed capillary tubes were regarded as being of exceedingly small diameter so that water could rise in them to considerable heights. It was commonly estimated, for example, that water could rise to the surface from an underground water-table as much as 30 feet

below, and the fact that rises of more than 3-4 feet could not be obtained in laboratory experiments was comfortably explained away by assuming that the field structure could not be reproduced by soil packed in tubes.

This interpretation necessarily implied that in a period of drought, crop roots well above the water-table were supplied with moisture which ascended by capillary action. On the basis of the capillary theory, there was a simple explanation of the supposed effects of such common cultivation operations as harrowing, hoeing and rolling. Harrowing and hoeing had the effect of breaking up the capillary tubes close to the surface, and of covering their broken ends with a loose layer of soil. The normal capillary rise of water from below was thus interrupted and water which otherwise would reach the surface and evaporate would, in fact, be retained somewhere below. Regarded in this way, harrowing and hoeing tended definitely to conserve moisture.

The same theory ascribed an exactly opposite effect to the results of rolling. Rolling would compress the soil, and by making the capillary tubes narrower, enable more water to be drawn up for the benefit of the young crop.

Criticism of the capillary tube theory

The first question is whether, in practical soil conditions, the maximum height of capillary rise ever reaches the values suggested by the capillary tube theory. This was investigated at Rothamsted over 15 years ago, using cylinders 2 ft. in diameter and 6 ft. deep, closed at the bottom so that all percolation water was retained. The cylinders were placed in excavations, with their rims just above ground level and filled with sand, or with soil in its original layers. In each cylinder a pipe 2 in. in diameter open at top and bottom, afforded a means of measuring the depth of the ground-water-level or, as it would be called in the field, the water-table. (See Fig. 1.) The cylinders were left for several years for the contents to settle, before measurements of the ground-water-level changes were begun. As percolation water could not escape, the ground-water-level reached the soil surface in the winter. It receded in the spring and summer, with temporary rises after any rainfall that was heavy enough to provide percolation water. By fitting together the receding portions of the curves, it was possible to construct a curve showing how the water-level would fall when

drought conditions were maintained at the soil surface for an indefinite period, i.e. conditions in which the so-called capillary rise would have maximum opportunity of action. It was fortunate, for this purpose, that the experiments included 1921, the year of the great drought. The results are shown in Fig. 1.

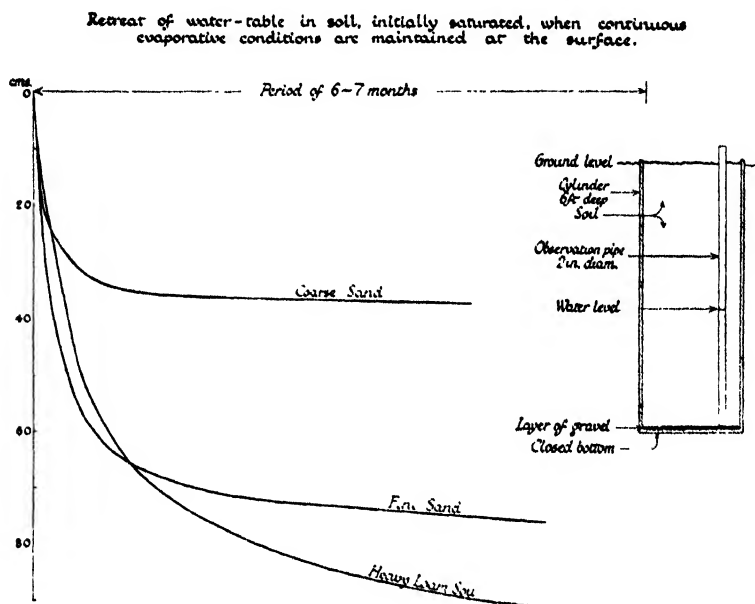


FIG. 1.

It will be seen that once the water-level had fallen quite a short distance below the surface (35 cm. and 70 cm. for coarse and fine sand respectively, and about 90 cm. for Rothamsted soil), it remained practically constant. The experiments therefore did not confirm the capillary tube theory for, according to the latter, water should have continued to rise by capillary action and, in consequence, the ground-water-level should have continued to fall. Putting the matter more accurately, these experiments, and a great many others that have been made from time to time at different centres, suggest that although a water movement in soil by capillary action may take place, it is limited, in practice, to relatively small distances. A rise of six feet would probably represent an extreme limit and, since over the greater part of the country the water-table lies considerably more than this distance

below the lowest root level of most plants, capillary action cannot in practice exercise much influence on growing crops.

Even where the water-table is near to the surface there are serious objections to the capillary theory. For one thing, in its simple form it requires that all the soil subject to capillary action should be uniformly saturated with water: a condition which obviously does not hold in practice. Nor have attempts ⁽³⁾ at getting over this difficulty without altering the basic theory—for example, by assuming that the tubes vary in diameter—been successful.

The modern theory of soil-water movement

As early as 1897 Briggs ⁽³⁾ in America broke away from the capillary tube theory, and developed a new method of approach, in which the water was considered to be spread in films over the surfaces of the soil particles. Although this was an improvement it still did not give the correct picture. The problem remained unsolved until the recent work of Haines ⁽⁴⁾ and Schofield ⁽⁵⁾ at Rothamsted. It is not necessary here to discuss the scientific aspects of this recent work. It is sufficient to remark that, instead of an assemblage of capillary tubes, modern workers regard the pore-spaces in soil as a series of cells communicating with one another by relatively narrow necks. A general picture of soil-water movement according to these studies can, however, be given.

Consider a soil whose moisture content has been reduced to a low value, and on which rain then falls. The particles in the upper layers are grouped into aggregates or crumbs that characterize 'tilth'. These crumbs are permeated by minute interstices and behave towards the rain-water much as a sponge. Any water in excess of the amount these crumbs can imbibe passes on downwards through the larger and cell-like pore-spaces between them, and continually decreases in amount as each successive layer of crumbs levies toll on it. In the subsoil and lower depths, where there is little or no crumb structure, the water is held mainly as little rings around the points of contact of particles and also in partially filled cell-like pore-spaces. (It is here assumed for simplicity that the lower depths are permeable.) Any percolation water surplus to the above requirements eventually reaches the underground water-table. The quantity of water that the soil will absorb is, in general, greater the higher the content of clay

and organic matter; but there is a fairly characteristic value for each soil which may be regarded as the maximum water-holding-capacity under field conditions. Since only the water that is surplus to the maximum water-holding capacity of any given layer reaches the next lower layer, a relatively light rain shower may be held entirely in the upper layers of soil. On the capillary tube theory this moist layer should share its water content with the drier layers immediately below, but both experiment and modern theory show that it does not. Any bulk movement is so slow as to be negligible for practical purposes, and the water held by the soil can be regarded as being relatively static. The modern theory shows that it resists movement and that evaporation from the surface proceeds by the progressive drying out of the top layers of soil rather than by upward movement of water from below to the surface. Similarly, absorption by plant roots does not cause water to move over any considerable distance into the depleted zone; the moisture remains relatively stationary, and it is only the fact that the plant's ramifying root system traverses the soil very completely during its growth that ensures that the root-hairs are never far away from a supply of moisture. The roots of common agricultural crops, if conditions permit, range much deeper than is generally supposed; typical figures are: wheat, oats, sugar beet 5-6 ft., barley 3-4 ft., potatoes 3 ft. The water-holding-capacity of a 5-ft. depth of soil is equivalent to at least $7\frac{1}{2}$ inches of rain, which is sufficient to meet the average transpiration requirements of a typical crop.

With regard to upward movement of water, the modern theory indicates that it takes place, if at all, over limited distances, and at a slow rate. A value of 3-4 ft. under field conditions is a generous estimate, as is indeed shown by the curve for a soil in Fig. 1. If, therefore, we take the maximum root range to be 5 ft., a water-table more than 3-4 ft. below that, i.e. 8-9 ft. below the soil surface, is not able, in practice, to supply any appreciable quantity of water for the plant (*), nor, in fact, does the plant require it. The water in the soil, together with that portion of the rain that is not re-evaporated, is sufficient. If this were not so it would be difficult to explain why the depth of the underground water-table is not sharply marked out by the appearance of crops during a drought. As has already been pointed out the water-table over a great part of arable Britain is many feet below the

surface, yet the controlling factor in drought resistance is not this depth, but the heaviness of the soil.

The bearing of the new theory of water movement on cultivation

It is clear from the foregoing discussion that earlier ideas about the action of harrows, hoes and rolls will need considerable revision—at any rate so far as their effect in controlling soil moisture is concerned. We cannot, in fact, expect cultivation operations to give any *direct* control at all in ordinary circumstances. Except when the water-table is very near the surface there will be no general upward movement of water, and evaporation from the surface will be confined to a progressive drying-out of the upper layers of soil. In these circumstances, mulching by harrows or other cultivating implements cannot conserve moisture—indeed by exposing a greater surface to the action of sun and wind it may even hasten the drying which takes place. When, on the other hand, the water-table is close enough to the surface for upward movement of water to take place, the movement is likely to be too slow to make good the loss by evaporation. The top layers will dry out and the resistance to diffusion of water evaporated from below will be increased. The rate at which water is lost from the surface will thus decrease until a state of affairs is reached in which the upward movement from the water-table is just able to make good the loss by evaporation. The soil, in fact, will become ‘self mulched’ and, in most cases, no more effective result would be achieved by mechanical mulching with implements. On the other hand, by removing the competition of weeds, harrowing or hoeing can conserve moisture indirectly. It may also be of definite value in some soils in breaking up a cap which might otherwise cause root damage in a young plant. But neither of these effects is concerned with moisture conservation in its ordinary sense. It would appear, too, that earlier workers’ ideas on the useful effects of rolling were also exaggerated. As we have seen, if the supposed action of the roller in increasing capillary action and bringing up a further supply of moisture could take place at all it would only be when the water-table was near the surface and the upper layers were saturated with water. And in these circumstances no farmer of experience would think of using a roll—indeed, to do so would almost certainly be disastrous. Any action, therefore, which the roller may appear to have in

bringing moisture to a young plant is almost certainly confined to pressing already moist soil around the plant so that the, as yet, partly developed roots can more easily extract the moisture.

In conclusion, it might be pointed out that if cultivation operations had any more direct action than those indicated above—if, in fact, they exercised anything like the delicate control of soil moisture commonly attributed to them—it would be impossible to account for the great mass of negative results that cultivation experiments have so far given. For moisture supply is obviously the greatest controlling factor in crop growth. Yet the extensive series of experiments, mainly on deep and shallow ploughing and subsoiling done in the United States ⁽⁷⁾; the work at Rothamsted comparing rototillage with traditional methods, and on intensive surface cultivations ⁽⁸⁾; the critical studies of standard cultural operations made at Cambridge ⁽⁹⁾ all show that the yield of crops is surprisingly insensitive to variations in cultural methods. Effects on early growth, often of a striking nature, are not infrequent, but they disappear, almost invariably, before harvest. It would seem that the only essential purpose of cultivation operations is to remove the competition of weeds for moisture—and many of the Rothamsted experiments cast doubt even on this; to facilitate the distribution of fertilizers; and to bring the soil into the loose mechanical condition necessary to create a seed-bed. If this be so, there are grounds for believing that an appreciable reduction in the number of operations, or a substitution of combined operations by new implements for the traditional sequence, could be made without any ill-effects either on crop yields or on the accepted standards of good husbandry, and with an appreciable reduction in production costs.

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CCXXIV. DETERMINATION OF THE FURFUR- ALDEHYDE YIELD OF SOILS AND OF PLANT MATERIALS ADMIXED WITH SOIL

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(Received 19 August 1937)

THE procedure usually employed for the estimation of the furfuraldehyde yield of plant materials is the phloroglucinol method of Kröber [A.O.A.C. 1935], but this method is time-consuming and the materials required are rather costly. Powell & Whittaker [1924] recommended the bromine titration method as giving consistent results which agreed with those obtained by the phloroglucinol procedure. The bromine method takes a much shorter time (about an hour) and the materials required are more easily available.

A preliminary comparison of the two methods showed that the bromine method gave good agreement with the phloroglucinol procedure for plant materials (Table I).

Table I. *Furfuraldehyde yield from plant materials*

Plant material	Amount taken mg.	Furfuraldehyde yield by	
		Bromine titration mg.	Phloroglucinol precipitation mg.
(1) Rice straw	400	53.2	52.1
(2) Oat straw	300	49.4	48.7
(3) Barley straw	300	49.8	49.1
(4) Ragi straw	300	54.1	53.2
(5) Grass (lawn mowings)	500	38.4	37.6
(6) Bracken leaves	1000	42.2	41.1

An examination of the applicability of either method to soils and materials admixed with soil raised several difficulties. The first lay in the procedure for distillation itself. It was found highly inconvenient to follow Kröber's procedure of adding 30 ml. portions of 12% HCl every 10 min. directly to the distilling flask, on account of the excessive bumping of the soil mass. This was avoided by taking 700 ml. of 12% HCl in a conical flask and distilling it into a 300 ml. distilling flask containing the soil material mixed with 50 ml. of 12% HCl, at such a rate that 500 ml. were collected in 2½ hr. This volume of distillate was found sufficient to collect almost the whole of the total furfuraldehyde yield obtainable from the material concerned.

The second difficulty met with was the adverse effect of the presence of soil on the yield of furfuraldehyde obtained. Considerably lower values were obtained in most cases (*vide* Table II) in presence of soil than in its absence, as has already been reported elsewhere [Acharya, 1937].

Table II. *Influence of soil on the estimation of furfuraldehyde yield*

Soil added	Furfuraldehyde yield in mg. (values for the soils only have been deducted in each case)		
	Rice straw 0.4 g.	Wheat straw 0.4 g.	Barley straw 0.4 g.
(1) Control value without the addition of soil	55.2	69.6	65.4
(2) 5 g. Paddy soil from Aduturai (India)	38.4	52.8	49.2
Do. ignited	32.4	46.2	43.8
(3) 5 g. Paddy soil from Coimbatore (India)	43.2	58.2	54.0
Do. ignited	36.6	52.2	47.6
(4) 5 g. Woburn soil (sandy loam) (England)	52.2	66.0	62.4
Do. ignited	45.6	59.4	56.4
(5) 5 g. Woking soil (sand)	55.2	69.6	65.4
Do. ignited	55.2	69.6	65.4

The results presented in Table II are typical of those obtained by an examination of a large number of soil samples. Heavy soils generally showed a greater interfering effect in the estimation of furfuraldehyde than light and sandy soils, and the adverse effect increased with the proportion of soil to plant material taken. This interfering effect was not due to the organic matter fraction of the soil (e.g. lignin), as shown by the fact that ignition of the soil not only failed to remove, but increased the harmful effect.

A detailed examination of the nature of the interference of soil in the estimation showed it to be due to the presence of oxidizing compounds in the soil, e.g. ferric and manganese compounds and nitrate (*vide* Table III). Bengtsson [1936] has noticed that in presence of nitrate lower values are obtained for furfuraldehyde and has recommended that in such cases the material should be washed with water to remove the nitrate before distilling for furfuraldehyde. Such a treatment, however, when applied to the soils examined, instead of improving the recovery of furfuraldehyde, actually tended to lower it, showing that the oxidizing action of soil is due only in minor degree to the nitrate content, but is brought about mainly by the inorganic mineral constituents, such as ferric and manganese compounds.

Table III. *Influence of oxidizing agents on the recovery of furfuraldehyde*

Substance added	Furfuraldehyde yield in mg.		
	Furfuraldehyde solution	Rice straw 0.4 g.	5 g. Aduturai soil and 0.4 g. of rice straw
(1) Control value without any addition	60.6	68.4	52.8
(2) Ferrous sulphate, 1 g.	60.6	68.4	52.8
(3) Ferric alum, 1 g.	58.2	57.0	46.2
(4) Ferric chloride, 1 g.	56.4	40.2	36.0
(5) Ferric oxide, 1 g.	55.2	24.0	22.8
(6) Manganous chloride, 1 g.	60.6	68.4	52.8
(7) Manganese dioxide, 1 g.	Nil	9.0	8.6
(8) Sodium nitrate, 1.2 mg.	58.2	65.4	49.2

As the removal of the interfering constituents could not be effected by water, an attempt was made to reduce the compounds. Various reducing agents such as Sn and HCl, Zn and HCl, Devarda's alloy in alkaline medium were tried, but the best was found to be the addition of stannous chloride (10% solution in HCl). This was therefore added to the soil in all distillations, and a comparison of the bromine titration and phloroglucinol methods was made.

Table IV gives the influence of adding different amounts of stannous chloride on the yield of "apparent furfuraldehyde", as determined by the two methods. The figures show that whereas the bromine value increases rapidly with increasing amounts of stannous chloride added, the phloroglucinol value first rises to a steady level and then shows an actual decrease. That the increase in bromine value was not due to a portion of the added stannous chloride being carried over with steam into the distillate was shown by running suitable blanks.

Table IV. *Influence of the amount of stannous chloride added on the recovery of furfuraldehyde from soils*

Amount of stannous chloride added	Furfuraldehyde yield by		
	Bromine titration method mg.	Phloroglucinol method	
		Before alcohol treatment mg.	After alcohol treatment mg.
(1) Rice straw, 0.4 g. only	53.2	52.1	51.8
(2) Aduturai soil, 5 g. and 0.4 g. rice straw, without stannous chloride	35.3	34.7	34.2
(3) Do. with 5 ml. SnCl_2	53.6	52.8	52.1
(4) Do. with 10 ml. SnCl_2	58.1	52.2	51.0
(5) Do. with 15 ml. SnCl_2	62.6	46.4	40.1
(6) Do. with 25 ml. SnCl_2	67.2	38.2	26.5
(7) Blank with 25 ml. SnCl_2 only without added material	Nil	Nil	Nil

The increasing solubility of the phloroglucinol precipitate in alcohol, at higher concentrations of stannous chloride, suggested the formation of methylfurfuraldehyde under these conditions [cf. Fenton & Gostling, 1901]. An examination of the behaviour of non-pentose carbohydrates such as glucose, starch and cellulose in presence of stannous chloride confirmed this view (Table V). Apparently, in the case of straw, the excess of stannous chloride should have similarly interacted with the non-pentose materials therein. Methylfurfuraldehyde combines with bromine similarly to furfuraldehyde, and it is difficult to distinguish between the two or to determine their relative proportions by this method. With phloroglucinol, however, methylfurfuraldehyde is partially precipitated as a brown precipitate which is readily soluble in hot alcohol. It is therefore possible to separate the two compounds and obtain correct values for furfuraldehyde only by treating the combined precipitates with boiling alcohol.

Table V. *Influence of SnCl_2 on the distillation of non-pentose materials with 12% HCl*

Added 10 ml. of 10% SnCl_2 and distilled. Controls without SnCl_2 deducted in each case	Apparent furfuraldehyde yield by		
	Bromine titration method mg.	Phloroglucinol method	
		Before alcohol treatment mg.	After alcohol treatment mg.
(1) Glucose, 1 g.	48.4	35.3	Nil
(2) Soluble starch, 0.5 g.	44.1	33.7	Nil
(3) Filter paper, 1 g.	22.2	15.1	Nil
(4) Oat straw cellulose, 0.5 g.	31.4	23.2	Nil
(5) Lignin, 0.5 g.	Nil	Nil	Nil

When there is only a slight excess of stannous chloride present beyond what is necessary to reduce the oxidizing substances contained in the soil sample taken, the bromine and phloroglucinol methods give concordant results. With larger amounts of stannous chloride up to nearly twice the required amount, the phloroglucinol value remains stationary while the bromine value steadily increases and the distillate becomes coloured yellow (*v.* Table IV). This difference between the two methods is apparently due to the fact that within this range the amount of methylfurfuraldehyde formed is comparatively small (about 10% of the furfuraldehyde produced) and is but partially precipitated by phloroglucinol. Even the small amount that may be so precipitated can be removed by treatment with boiling alcohol. With larger amounts than double the required quantity, however, the effect of stannous chloride is to depress the amount of furfuraldehyde formed and increase that of methylfurfuraldehyde and its volatile decomposition products. Hence the bromine value continues to increase, while the phloroglucinol value decreases, and the residue after alcohol treatment shows a still more rapid decrease.

Usually, a quantity of 10% SnCl_2 corresponding to 1 ml. per g. of soil taken is found to be a good approximation to the quantity required for reduction of oxidizing substances present; and the distillates obtained are colourless. Sandy soils require rather less, whilst clayey soils require relatively more. As it is difficult, however, to judge beforehand the exact amount of SnCl_2 to be added in each case, and a slight excess vitiates the bromine value, but does not affect the phloroglucinol value, it is concluded that for soils and plant residues mixed with soil, distillation of the material with 12% HCl in presence of stannous chloride and estimation of the furfuraldehyde evolved by precipitation with phloroglucinol, followed by extraction of the precipitate with boiling alcohol, is preferable to the bromine titration method of Powell & Whittaker [1924].

SUMMARY

1. The present paper reports a comparison of the bromine titration method of Powell & Whittaker and the gravimetric phloroglucinol method for the estimation of the total furfuraldehyde yield of soils and plant materials admixed with soil. In the absence of soil, the two methods were found to give concordant results.

2. In presence of soil, however, low results were obtained by both the methods, owing to the presence of oxidizing agents such as ferric and manganese compounds and nitrate in the soil, which apparently oxidize a portion of the furfuraldehyde during the course of distillation with 12% HCl . The addition of stannous chloride in regulated amounts serves to reduce such oxidizing agents and yield the proper recovery of furfuraldehyde. About 1 ml. of 10% SnCl_2 per g. of soil was found to be sufficient for most soils and to leave a slight excess.

3. This excess of stannous chloride, however, tends to produce methylfurfuraldehyde, which reacts with bromine and increases the "apparent" furfuraldehyde yield as determined by bromine titration. The interference is much less in the case of phloroglucinol, as methylfurfuraldehyde is only partially precipitated by this reagent and even this interference can be removed by treating the combined precipitate with boiling alcohol, in which the impurity is easily soluble. A large excess of stannous chloride, however, gives a coloured distillate and depresses the formation of furfuraldehyde.

4. Hence, it is concluded that for soils and plant materials mixed with soil, distillation of the material with 12% HCl in presence of stannous chloride and

estimation of the furfuraldehyde by precipitation with phloroglucinol, followed by extraction of the precipitate with boiling alcohol, is preferable to the bromine titration method of Powell & Whittaker.

In conclusion, the writer desires to thank Sir John Russell and Mr E. H. Richards of the Rothamsted Experimental Station, England, and Prof. V. Subrahmanyam of the Indian Institute of Science, Bangalore, for facilities accorded to carry out the investigation.

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THE OXYGEN UPTAKE OF SUSPENSIONS AND CULTURES OF A FREE-LIVING BACTERIUM

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(Received 21 September 1936)

(With Four Text-figures)

INTRODUCTION

THE study of the metabolic processes taking place in a growing culture of micro-organisms presents some particular difficulties, and, in consequence, comparatively little work along these lines has as yet been reported. Among earlier papers on this subject, the following may be mentioned. The course of events during the growth cycle of a single culture has been investigated by Walker & Winslow and their associates (1932, 1934), who measured the carbon dioxide output and ammonia production (from peptone) of a pure culture of *B. coli*. Martin (1932), using the same organism, has made continuous studies of the oxygen uptake throughout its growth cycle; he found a pronounced maximum in the oxygen uptake per cell, occurring at the end of the lag period, or early in the logarithmic growth period, which he was able to associate with the time of maximum cell size. Burk & Line-weaver (1930), in the course of their intensive studies on the metabolism of *Azotobacter*, compared the constant rate of respiration of a non-growing culture (deprived of nitrogen) with the increasing rate of a growing culture.

In the study of the metabolism of a growing culture, the use of a free-living organism, which can be grown at a temperature of 25° C. or so, presents certain definite advantages over the use of a pathogenic strain. As the growth cycle is spread over a longer period, 4-6 days, instead of as many hours at 37°, it is possible to obtain a more detailed picture of the events at each stage of growth.

In the present study, the oxygen uptake of a free-living bacterial species, derived ultimately from soil, and used in pure culture, was studied by means of Barcroft differential manometers. Two sets of experiments were carried out; in the first set, single observations were made on small amounts of liquid medium heavily inoculated with bacteria; these are referred to as *suspension* experiments throughout this paper. In the second set, referred to as *culture* experiments, a large volume of medium was seeded with a small inoculum of the bacteria in each case, and the resulting culture was observed at intervals up to the peak stationary phase of growth.

Description of species

The bacterial species employed in these experiments was one of a number isolated, in the course of investigations on the purification of effluent from a beet-sugar factory, by the General Microbiology Department at Rothamsted. It was obtained from the film on a gravel filter used in this work, and was probably originally derived from the soil washed off the beets, which was the principal source of inoculation of the filter. In the present state of classification of the saprophytic bacteria, it is impossible to assign a specific name to it; it resembles in some respects the description given in Bergey's *Manual* (1934) for *Achromobacter fermentationis* (Chester), but differs from it in not coagulating milk in 7 days. Its cultural characteristics are as follows: rods— $1.0\mu \times 0.8\mu$, non-motile, non-spore forming, gram negative; agar streak—growth good, filiform, white, opalescent, smooth, edge undulate, medium unchanged; colonies on Thornton's agar—round, white, slightly convex, opalescent. Does not liquefy gelatine. Produces acid and gas on sucrose and levulose, acid on dextrose, maltose and mannitol, acid and gas on glycerine. Reduces nitrates to nitrites. Forms acid on litmus milk, no coagulation or digestion. Aerobic, facultative.

Method of counting

The bacteria were counted throughout by the direct method, using a Thoma haemocytometer, 0.02 mm. deep, with $1/400$ mm.² squares. Duplicate counts were made in every case. This method of counting includes all bacterial cells present, whether viable or not.

Measurement of oxygen uptake

The apparatus used for the measurement of oxygen uptake consisted of Barcroft differential manometers; the technique employed has been fully described by Dixon (1934). The volume of sample used was 3 c.c. of the liquid culture, or suspension, in every case. The measurements were made at a temperature of 26° C., and KOH solution on filter paper was used to absorb carbon dioxide.

Estimations of the respiratory quotient (carbon dioxide evolved/oxygen absorbed) were made by the technique described by Dixon (1934), in which one manometer is used to measure oxygen uptake, one (without KOH paper) to measure the difference between oxygen uptake and carbon dioxide evolution, and a third to measure the amount of bound carbon dioxide in the medium. This method gives an approximate estimate only of the respiratory quotient.

I. SUSPENSION EXPERIMENTS

METHODS

In these experiments, heavy suspensions of young cells were made by washing off the surface growth from cultures on a solid medium into a liquid medium containing either alanine (carbon and nitrogen both supplied) or glucose (carbon only).

The bacteria were grown on nutrient agar; one, two or three tubes of this medium (according to the thickness of suspension desired) were inoculated and grown at 22° C. for either 24 or 48 hours. 0.5 c.c. of the liquid medium was then poured on to the surface

of each slope with a sterile pipette, and the surface growth emulsified in this liquid with a loop; the emulsion was pipetted into 10 c.c. of sterile medium to form the suspension used. The number of bacterial cells in the suspension was counted in duplicate at the beginning and end of the experiment. The oxygen uptake of two samples of the suspension was measured over a period of 6 hours.

Media

The following liquid media were used:

(a) *Glucose medium* (contains no nitrogen).

0.2 per cent glucose and 0.12 per cent KH_2PO_4 in a mineral salt solution of the following composition: NaCl 0.06 per cent, KCl 0.001 per cent, CaCl_2 0.002 per cent, MgSO_4 0.001 per cent. Steamed for 1 hour, pH adjusted to 7.2 with N/10 NaOH, steamed again for 1 hour.

(b) *Alanine medium*.

As above, except that the glucose was replaced by 0.2 per cent of alanine (α -amino-propionic acid).

RESULTS

The results obtained from twelve experiments with the alanine medium showed two constant differences from the ten experiments with the glucose medium, which are illustrated in Table I. This table summarizes the results from six typical experiments, arranged in pairs, each pair consisting of one alanine suspension and one glucose suspension, with approximately equal bacterial numbers at the start of the experiment.

Table I

Time from start (hr.)	Alanine		Glucose	
	Bacterial nos. (millions per c.c.)	Rate of oxygen uptake per $\frac{1}{2}$ hour (mm. ³ from 3 c.c.)	Bacterial nos. (millions per c.c.)	Rate of oxygen uptake per $\frac{1}{2}$ hour (mm. ³ from 3 c.c.)
1st pair				
0	462	—	434	—
0- $\frac{1}{2}$	—	15	—	6
3-4	—	55	—	9
5-6	—	94	—	7
6	1200	—	380	—
2nd pair				
0	884	—	926	—
0- $\frac{1}{2}$	—	35	—	39
3-4	—	80	—	33
5-6	—	150	—	27
6	2240	—	1163	—
3rd pair				
0	1026	—	1060	—
0- $\frac{1}{2}$	—	49	—	36
3-4	—	112	—	29
5-6	—	161	—	27
6	2660	—	1038	—

The first difference between the suspensions on alanine and those on glucose is that a considerable increase in bacterial numbers takes place on the first medium, but not on the second. The second difference is that the rate of oxygen uptake rises markedly and continuously in the alanine suspensions, but falls a little with time in the glucose suspensions. This is further illustrated in Fig. 1, which shows the rate of oxygen uptake for each half hour during the whole course of the second pair of experiments in the table. The curve obtained from the alanine suspension is concave upwards; a similar curve was obtained from all the alanine experiments, and its nature can be seen if the logarithms of the respiration rates are plotted against time. Fig. 2 shows the logarithms of the rates of oxygen uptake derived from two alanine experiments (4 and 9). The observed points in experiment 4 fall very nearly on a straight line; the rate of oxygen uptake is increasing logarithmically throughout. In other cases, however (as in experiment 9), there seems to be a preliminary period of adjustment, after which the rate increases logarithmically.

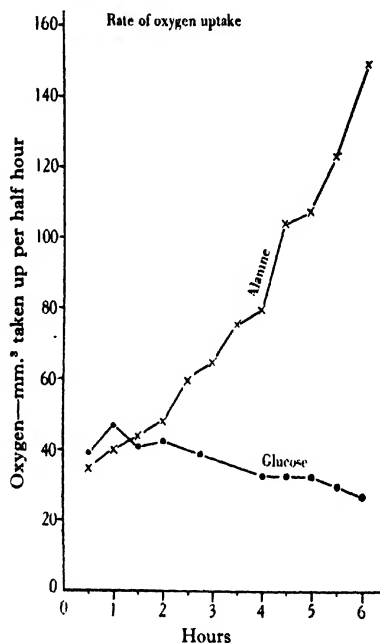


Fig. 1. The rates of oxygen uptake per half hour in a pair of suspensions containing about 900 million bacteria per c.c. at the start.

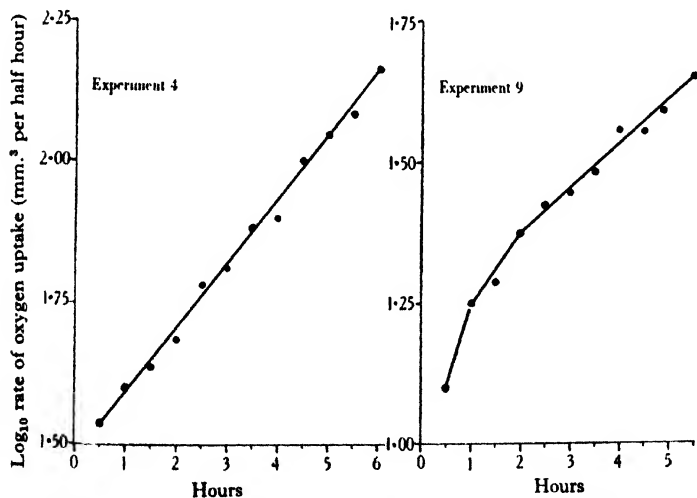


Fig. 2. Logarithms of the respiration rates in two suspensions on alanine.

RELATION BETWEEN GROWTH AND INCREASE IN RESPIRATION RATE

As the increase in respiration rate has been used by Burk & Lineweaver (1930) as a measure of bacterial growth, it is of interest to see whether in these experiments the increase in the rate of oxygen uptake corresponds with the increase in bacterial numbers. If the respiration rate increases during the experiment in proportion with the increase in bacterial numbers, then the rate of respiration per cell should be the same at the end of the experiment as at the beginning. In Table II are given the rates of oxygen uptake per $\frac{1}{2}$ hour from 3 c.c., and the rate per hour per 1000 million bacteria for the first half hour and the last half hour of eight experiments on the alanine medium.

Table II

Exp.	Start			Finish		
	Number of bacteria (millions per c.c.)	Rate per $\frac{1}{2}$ hour (mm. ³)	Rate per hr. per 1000 million	Number of bacteria (millions per c.c.)	Rate per $\frac{1}{2}$ hour (mm. ³)	Rate per hr. per 1000 million
1	1026	49	34	2660	161	41
2	462	15	31	1200	94	49
3	520	20	30	1508	95	40
4	884	35	28	2240	150	41
5	1302	45	23	2460	137	37
6	450	22	36	1445	90	45
7	834	34	30	1970	154	48
8	624	32	38	1980	157	49

In every case the rate of oxygen uptake per 1000 million cells is larger at the end of the experiment than at the beginning; the increase in respiration rate is greater proportionately than the increase in bacterial numbers. It is possible to consider the total oxygen uptake of such an actively growing suspension as divisible into two parts—(a) “maintenance” respiration, directly proportional to the number of bacteria present in the suspension, and therefore increasing during the experimental period, but insufficient to account for the total increase in respiration rate of the culture; and (b) “growth” respiration in the strict sense, presumably proportional to the division rate of the cells at any time.

Respiratory quotient

Nine estimations of the respiratory quotient were made on the glucose, and nine on the alanine medium; the R.Q. in both cases was a little less than unity. The glucose experiments gave a mean value of 0.88 (standard error ± 0.013), and the alanine experiments a mean value of 0.93 (standard error ± 0.017).

II. CULTURE EXPERIMENTS

METHODS

In these experiments an effort was made to follow the course of events during a complete growth cycle, in cultures started with a small inoculum, by successive counts, and measuring the oxygen uptake of samples taken at intervals up to 24 hours apart.

Medium

The medium used throughout was the alanine medium described in section I; 60 c.c. of this liquid medium, in a sterile 250 c.c. conical flask, were used in each experiment.

Inoculations

Two loopfuls of the growth from a young agar slope culture (18–30 hours old, usually 24 hours) were emulsified in 10–20 c.c. of sterile medium in a small flask. The cells in the resulting suspension were counted with a haemocytometer, and 1–3 c.c. of the suspension were added to the culture flask, to give an initial count of between 5 and 10 million cells per c.c. This method has been found to give more consistent results than direct inoculation of the culture flask.

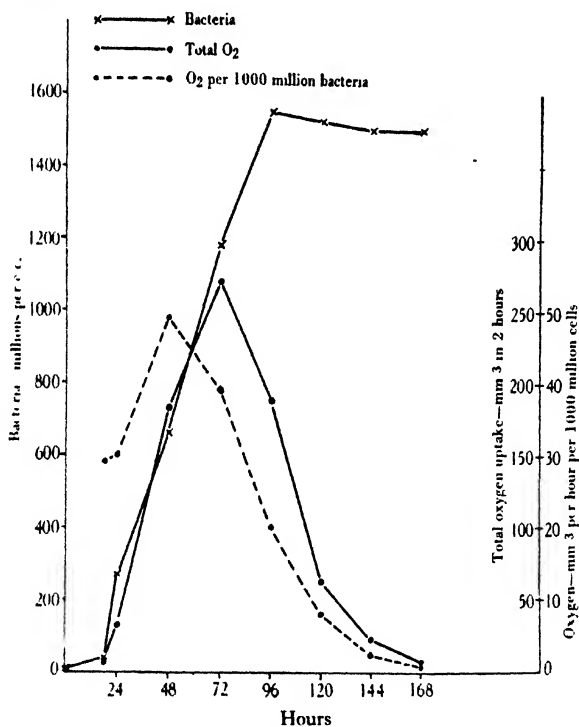


Fig. 3. Course of events in a growing culture.

Sampling

The culture flasks, plugged with cotton wool, were suspended in a water bath at a temperature of about 25° C. ($\pm 0.5^\circ$). No attempt was made to aerate the cultures. They were maintained for periods up to 7 days from the inoculation, and, at daily or shorter intervals, samples were withdrawn, the numbers of bacteria in them counted, and their oxygen uptake measured over a period of 2 hours.

RESULTS

The course of events occurring in a culture under the given conditions is illustrated by Fig. 3, which represents the average figures derived from six experiments performed under identical conditions, and giving closely similar results. It

will be seen that the numbers of bacteria present in the cultures increased up to 96 hours after inoculation, when a steady state was reached at about 1500 million cells per c.c. The total oxygen taken up in a 2-hour period attained a maximum value of about 270 mm.³ in samples taken from 72-hour-old cultures; samples from older cultures gave values for total oxygen uptake decreasing rapidly with age, till, a week after inoculation (and 3 days after the stationary state was attained), the oxygen uptake was so small as to be only just measurable. Similar results were obtained for CO₂ output by Walker & Winslow (1932). The oxygen uptake per cell (calculated as the oxygen uptake per hour per 1000 million bacteria) reaches a maximum value in samples taken 48 hours after inoculation, during the early part of the logarithmic growth period. On account of the smallness of the organism employed, it was impossible to measure any but the grossest variations in the size of individual cells; large variations in size were not observed, so the maximum value of oxygen uptake per cell cannot be definitely connected with the time of maximum cell size (cf. Martin, 1932).

RESPIRATORY QUOTIENT

A few estimations of the respiratory quotient, made at different times in the growth cycle, gave values of unity or slightly below in nearly every case. The figures are given in Table III, which also shows the progressive increase, with the age of the culture, in bound CO₂ present in the medium.

Table III. *Respiratory quotient and bound CO₂*

Age of culture	24 hours	48 hours	72 hours	96 hours
R.Q. (av.)	0.95	1.0	0.97	0.91
Bound CO ₂ (av.) (mm. ³ in 3 c.c.)	75	134	233	340

COURSE OF OXYGEN UPTAKE IN INDIVIDUAL SAMPLES

The course of oxygen uptake in 2 hours from samples taken at intervals of 24 hours in a single experiment is illustrated in Fig. 4, which gives the rate of oxygen uptake for each $\frac{1}{2}$ hour (mean values from duplicate samples). The oxygen uptake of the sample taken 24 hours after inoculation is very small, and proceeds at a nearly constant rate. On the other hand, the large oxygen uptakes of the 48- and 72-hour samples proceed at a continually increasing rate. When the stationary state is reached, at 96 hours, the sample still takes up a considerable amount of oxygen, but the rate of uptake is constant, as it is in the 120- and 144-hour samples (allowing for irregularities due to the larger error in reading the manometers when the rate is so small).

The 96-hour and subsequent samples therefore resemble the glucose suspensions, showing a constant rate of oxygen uptake associated with an absence of bacterial growth; in the samples taken when the bacteria are actively growing, the oxygen is absorbed at a continuously increasing rate, as in the alanine suspensions.

SHAPE OF THE CURVE OF OXYGEN UPTAKE

The 2-hour experimental period used in the earlier experiments is too short to estimate the shape of the rising curve obtained from samples taken in the logarithmic growth period, and for comparison with the suspension experiments a culture experiment was performed in which the readings were extended over 6 hours for each sample. The bacterial numbers, rates of oxygen uptake and the logarithm of

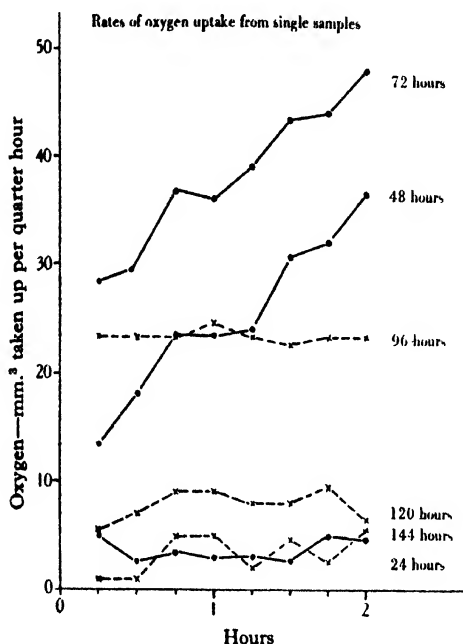


Fig. 4. Rates of oxygen uptake on successive days in a single experiment.

the rates in the 48-hour sample are given in Table IV. The other samples gave similar results.

Table IV. 48-hour sample

Time from start of readings (hr.)	Number of bacteria (millions per c.c.)	Rate of oxygen (mm.³ per ¼ hr.)	log ₁₀ rate	Difference
0	602	—	—	—
½-1	—	66	1·820	—
1-1½	—	75	1·875	0·055
1½-2	—	86	1·935	0·060
2-2½	—	97	1·987	0·052
4-4½	—	165	2·217	0·057
4½-5	—	181	2·258	0·041
5-5½	—	183	2·262	0·004
5½-6	—	194	2·288	0·026
6	1940	—	—	—

It will be seen that, for part of the six-hour readings, the rate of oxygen uptake is increasing logarithmically, as in the suspension experiments on alanine.

The readings taken for as long a period as 6 hours in a small sample do not, however, really represent what is happening in the original culture over the same period, as is shown by the bacterial numbers. In the Barcroft bottle, the numbers show a greater increase in 6 hours than is shown in 24 hours in the culture flask. A similar stimulation of bacterial growth in an apparatus which was shaken (in that case the Warburg apparatus) was observed by Wohlfeil & Ewig (1935). As a method of discovering the real course of events in the original culture, it would be better to take samples at frequent intervals, and to observe them in the Barcroft apparatus for the shortest time which suffices to obtain an adequate manometer reading.

EFFECT OF REMOVAL OF CELLS FROM A CULTURE

As has already been stated in the first section of this paper, it is possible to think of the oxygen uptake of a growing culture as divided into two parts—"maintenance" respiration and "growth" respiration. A series of observations made during the whole growth cycle of a culture, as illustrated in Fig. 3, suggests that a growth respiration proportional to the rate of cell division is superimposed on a maintenance respiration which falls as the culture gets older; the two together giving a peak value for oxygen uptake in the middle of the logarithmic growth period, before the numbers of bacteria reach their maximum value.

A method of estimating the relative size of these two components of the total oxygen uptake was found in the removal of cells from a culture in the logarithmic growth period; this compared with a control culture under the same conditions should give cultures of the same age (and therefore presumably at the same level of maintenance respiration), but with different growth rates. Two such experiments were performed, one at 25° C. and a second at 22° C. Two conical flasks *A* and *B*, each containing 60 c.c. of alanine medium, were inoculated at the same time from the same suspension; flask *A* was used as a control. In the middle of the logarithmic growth period, 48 hours after inoculation in the first case, and 72 hours in the second, samples were taken from both *A* and *B* for the estimation of bacterial numbers and oxygen uptake. Immediately afterwards, half the contents of flask *B* were removed, filtered through a Pasteur-Chamberland L3 candle to remove all bacterial cells, and replaced. Six hours later, and again 24 hours later, further determinations of bacterial numbers and the oxygen uptake during 2 hours were performed on samples from both *A* and *B*. The results are given in Table V.

It will be seen that the samples taken shortly after the removal of cells from flask *B* (at 54 and 78 hours respectively), show an increased oxygen uptake, relative to the number of cells present, as compared with samples from flask *A*. It will also be seen that the growth rate, as judged by the amount of growth recorded 18 hours later, is higher in flask *B* than in the control. Assuming that the maintenance respiration per 1000 million cells is the same in cultures of the same age, and assuming that growth in both cultures is proceeding logarithmically, it is possible to calculate the approximate values of maintenance and growth respiration in both

cases, for the samples taken at 54 and 78 hours (the next sampling after the removal of cells), since the total oxygen uptake is known in both cases.

Table V. *Effect of removal of cells*

Hours after inoculation	Bacteria (millions per c.c.)		Oxygen uptake (mm. ³ in 2 hours)		Uptake per hour per 1000 million cells	
	A	B	A	B	A	B
Exp. 1 (25° C.)						
0	11	11	—	—	—	—
48	864	*950	162	182	31	32
50	—	470	—	—	—	—
54	916	564	246	172	45	51
72	1332	1192	207	264	26	37
Exp. 2 (22° C.)						
0	4	4	—	—	—	—
72	616	*580	112	101	30	29
78	738	322	127	93	29	48
96	934	906	230	205	41	38

* Immediately after this count, half the cells in *B* were removed.

Experiment I

In culture *A* there are 916 million cells per c.c. at 54 hours after inoculation, and 1332 million at 72 hours. By interpolation an estimate of the growth during the 2 hours from 54–56 hours, when the oxygen uptake was being measured, gives a rate of growth of 20 million cells per hour—2·1 per cent of the calculated mean value (935 million). Similarly in culture *B*, starting with 564 million cells per c.c. at 54 hours, the growth rate over the next 2 hours is estimated at 23·5 million cells per hour—4 per cent of the calculated mean value (588 million). The total oxygen uptake from 3 c.c. of culture during the period from 54–56 hours after inoculation is 246 mm.³ in the *A* sample, and 172 mm.³ in the *B* sample.

Assuming that

x = maintenance respiration in mm.³ per hour per 1000 million cells,

y = growth respiration per 1 per cent growth,

we can then derive two equations in x and y from the data obtained from *A* and *B* respectively.

From *A* we have

$$\left(x \times 2 \times \frac{3 \times 935}{1000}\right) + (y \times 2 \cdot 1) = 246 \quad \dots\dots(1),$$

and from *B*

$$\left(x \times 2 \times \frac{3 \times 588}{1000}\right) + (y \times 4) = 172 \quad \dots\dots(2).$$

On solving these equations we obtain values for x and y as follows:

$$x = 41 \cdot 4,$$

$$y = 6 \cdot 60.$$

Experiment II

On considering the data derived from the observations made from 78–80 hours after inoculation in the second experiment, the growth rate in flask *A* during this period is estimated at 10 millions per hour, or 1.34 per cent of a calculated mean value of 748 million cells per c.c.; and in *B* it is estimated at 20 millions per hour, 5.89 per cent of the calculated mean value (341 millions per c.c.). From these figures, and the values obtained for the total oxygen uptake, we obtain the following equations:

$$4.49x + 1.34y = 127 \quad \dots\dots(3),$$

$$2.05x + 5.89y = 93 \quad \dots\dots(4).$$

The values of x and y which fit both these equations are

$$x = 26.3,$$

$$y = 6.63.$$

Though these values of x and y are approximate only, it is interesting that the same value for y should be obtained in both experiments, and that x , the maintenance respiration, should be smaller in experiment II, where the culture is older than in experiment I. Incomplete though these experiments are, they yet suggest a means of separating the effect of cell division on metabolism from the effect of other factors which change during the life of a culture in a limited volume of medium.

DISCUSSION

The most salient points that emerge from the results obtained on the course of oxygen uptake during a growth cycle in a culture are two in number. Firstly, the peak in oxygen uptake per cell occurring at 48 hours after inoculation (cf. Martin, 1932); and secondly the rapid drop in oxygen uptake in successive samples taken after the numbers of bacteria had reached their highest point. It is not possible, from the data obtained, to say whether the smallness of the oxygen uptake in the week-old culture is simply caused by exhaustion of the substrate, or whether other causes contribute to this effect.

The results of both sets of experiments show a very marked difference between growing bacteria and bacteria in a stationary state. In the stationary states observed, i.e. suspensions supplied with glucose but no nitrogen, and cultures which had reached their maximum density, oxygen was absorbed, sometimes in considerable quantity, at a constant rate. In suspensions supplied with nitrogen as well as carbon, and in cultures in the logarithmic growth phase, the rate of oxygen uptake increased continuously during the period of observation. It might be expected that, as the numbers of bacteria present are increasing from moment to moment, the oxygen uptake would also increase proportionately to the increase in numbers; but the results of the suspension experiments show that the increase in oxygen uptake was greater in proportion than the increase in bacterial numbers. This led to the supposition that the respiration of growing bacteria could be divided into "maintenance" and "growth" respiration, the former furnishing enough energy

to keep the cell alive, and the latter the extra energy required for cell division. The effect of removing cells by filtration from a culture in the logarithmic growth phase is to increase both the growth rate and the oxygen uptake per cell. Analysis of two such experiments suggests that growth respiration, per unit of growth, is constant during this logarithmic period, and that the maintenance respiration per cell falls as the culture grows older.

It may be of interest to note that the inverse relation between bacterial numbers and respiration per cell, which was observed in an earlier series of experiments on carbon dioxide output (Meiklejohn, 1932), did not hold good for the experiments reported in the present paper. A similar inverse relation, between bacterial numbers and oxygen uptake, was observed by Wohlfeil (1930); in both cases, the more bacteria present in a culture, the lower the respiration per cell; this relation was independent of the age of the culture. Both my former experiments, and those of Wohlfeil, used very different techniques to that employed in the present study; in both methods, air was bubbled comparatively slowly through a stationary flask. In a later paper, Wohlfeil & Ewig (1935) state that they find no evidence of this inverse relation in a series of experiments performed with the Warburg apparatus, which resembles the Barcroft apparatus in that the experimental material is shaken, and kept under conditions of maximum air supply. Similarly, the results reported in the present paper give no indication of an inverse relation between bacterial numbers and oxygen uptake per cell.

I should like to express my thanks to Mr D. Ward Cutler, in whose department this work was carried out, for his constant help and encouragement.

SUMMARY

1. The oxygen uptake of pure cultures and suspensions of a free-living bacterial species has been measured at 26° C.
2. At this temperature, liquid cultures reach the maximum stationary phase of growth about 96 hours after inoculation.
3. The greatest total oxygen uptake was observed in samples taken from cultures 72 hours after inoculation, and the greatest uptake per cell at 48 hours after inoculation. The oxygen uptake in cultures which have reached the stationary state falls off rapidly, at each successive sampling, to a very low value.
4. In suspensions deprived of nitrogen, and showing no growth, and in cultures in the stationary phase, oxygen uptake proceeds at a constant rate.
5. In both suspensions and cultures, where active growth is taking place, the rate of oxygen uptake rises continuously; after a preliminary period of adjustment this rise is logarithmic.
6. The rise in oxygen uptake in a growing suspension is proportionately greater than the rise in bacterial numbers.
7. It is suggested that the oxygen uptake of a growing culture can be divided into two parts; "maintenance" respiration and "growth" respiration, and a technique is outlined for estimating the amount of oxygen uptake due to each factor.

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SOIL PROTOZOA; THEIR GROWTH ON VARIOUS MEDIA

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THE methods at present used for the determination of the number and species of soil Protozoa are open to criticism, and it was with the hope of improving this technique that the present investigation was undertaken.

The usual method of using peptone agar as a medium for the growth of the Protozoa was criticized by François Perey(4) in 1925 when she made duplicate counts from a French garden soil, using peptone agar, and also an agar medium made from a decoction of the same soil. The numbers she obtained with the soil medium were higher than those from peptone agar. The numbers of flagellates, however, though higher were not significantly different; but the amoebae, *Naegleria gruberi*, *Vahlkampfia* sp., and *Hartmanella hyalina*, were increased. In the case of *Colpoda steinii*, the only ciliate recorded in her paper, the numbers were doubled. Figures were given for one sample: 1,193,000 Protozoa per gram of soil with soil extract agar, as compared with 56,400 Protozoa per gram of soil with peptone agar.

Similar results were obtained by Sandon(10) dealing with three Utah soils, and using agar in which soil or soil extract had been added in the place of mineral salts and peptone. The numbers obtained were higher than when the usual peptone agar was employed. Table I gives the numbers for these three alkaline field soils.

Table I
Alkaline field soil, Utah (Sandon)

	Flagellates		Amoebae		Ciliates	
	Peptone	Soil	Peptone	Soil	Peptone	Soil
A 2	Few	7193	28	115,000	Few	Few
R	225	1798	1798	14,386	28	450
B	57	57	900	3,596	7	57

A few experiments made on Rothamsted soil did not give these big differences, and as Sandon's results were obtained from alkaline soils and François Perey's from rich garden soil, it was decided to continue the investigation, using as many types of soil as possible to discover whether

peptone agar is generally the best medium for the qualitative investigation of soils. This was facilitated by receiving a varied collection of soils from the tobacco region in the south of the U.S.S.R., comprising 55 soils from eight different regions collected by the State Institute for Tobacco Culture.

EFFECT OF STORAGE

As these soils were all received in a dry state and the time required for examination extended over a long period the effect of storage must be taken into consideration. Very little work has been done on the effect of storage on the numbers of Protozoa found in the soil, when it is kept in a dry state. Fantham & Paterson⁽³⁾ in their work on South African soils showed that some species of Protozoa persist, though not all survive in soil bottled for 3 years. They state that ciliates from bottled soils develop more rapidly in cultures than those from fresh soil. Goodey⁽⁵⁾ also obtained an abundant protozoan fauna from soil stored for many years. Work in this department on stored soils⁽²⁾ showed that, if the water content is satisfactory, the organisms exhibit the same fluctuations as found in normal field soils.

The numbers of Protozoa found in the tobacco soils did not differ abnormally from those in soils examined 12 months later. It is reasonable to suppose that as these soils were collected in the dry hot season, in July and August, when the soils are dried in the sun and contain comparatively little moisture, the effect of storage on the numbers of Protozoa would be less than if the soil was collected in a damp condition. However, it is not claimed that the numbers of the Protozoa found are the exact figures, as they probably represent only the cystic forms found in the soil, and it was impossible while at the State Institute for Tobacco Culture to make any quantitative investigations into the numbers of active and cystic Protozoa in the soil.

METHODS

The total numbers of Protozoa were counted by using peptone agar, and soil extract agar made from that soil. This was made by boiling 200 g. of soil with 500 c.c. of tap water gently for 1 hour and filtering under pressure, after which the liquid was made up to 400 c.c., and 5 g. of agar were added. The medium was then sterilized in the autoclave, at a pressure of 15 lb., and allowed to cool immediately; the sterilization was completed by steaming on two successive days for 1 hour. Unpublished work done in this department suggests that if soil extract is heated for too long a period, it has a detrimental effect on the Protozoa;

the dilution plates were therefore poured while the agar was hot after the third sterilization, in order to avoid further heating.

The dilution counts, for the quantitative examination of the soil, were done by the method given by Cutler *et al.* (1). The Petri dishes were kept at room temperature, and examined at intervals of 7 days for 1 month, being moistened with sterile tap water when necessary.

PART I. U.S.S.R. TOBACCO SOILS

The total number of species from these soils recorded on soil extract agar was higher in every case than that on peptone agar, the average number on the former being 11 and on the latter 6. The average number of Protozoa is low, because it is calculated from all the successive samples in the same profile. The depths vary, from one region the samples being taken from the surface and at successive depths to 50–55 cm. and from another to 60–65 cm. These deeper samples, though never without Protozoa, yielded only a few species. With three exceptions the total number of Protozoa was much higher on the soil extract agar, but in these three cases, the flagellate *Cercomonas crassicauda* was present in much higher numbers on peptone agar than on soil extract agar. The numbers of *Oikomonas termo*, in one of these samples, were also higher on the peptone agar. With these exceptions more species and individuals were always found on soil agar plates. The examination of these three samples could not be repeated as there was not enough soil available.

Table II shows the differences in the numbers of species and individuals in the two agars in the first 12 soils examined from the U.S.S.R.

The soil samples referred to in Table II were taken from the surface to a depth of 10 cm., but most of the institutes sent soils taken at various depths, thus giving interesting figures for comparison. The figures show the big increase in the numbers of all classes of the Protozoa when soil extract agar is used, but it is most noticeable in the case of amoebae and, to a lesser degree, ciliates, which though usually found in the top layers of the soil do not readily develop on peptone agar. The differences in the ciliates are interesting since from 55 samples of soil, 40 recorded ciliates on soil extract and only five on peptone agar. The growth of flagellates on the two agars is very erratic, generally the numbers are higher on soil extract agar, but they are sometimes similar and, in three samples out of the 55 soils, the development is greater on the peptone agar. Two of these exceptions, samples 1 and 10, are shown in Table II. These high numbers are invariably due to the excessive growth of common forms.

Table II
Twelve Russian soils

Sample	Amoebae		Flagellata		Ciliata	
	Total no. per g.	No. of species	Total no. per g.	No. of species	Total no. per g.	No. of species
Soil extract agar						
1	59,268	8	187,874	10	118	4
4	960	3	2,783	10	22	2
7	1,321	7	2,746	12	17	2
10	21,250	2	8,402	8	175	2
13	121,938	6	433,159	8	86	2
16	1,901	4	737	8	15	1
20	3,710	2	3,656	8	32	2
24	3,625	5	45,017	9	40	2
29	117	2	2,597	5	0	0
31	2,600	1	377,185	9	22	2
36	3,620	4	10,010	8	38	1
41	1,322	3	75,254	7	38	1
Peptone agar						
1	10,407	2	370,429	8	0	0
4	560	2	2,161	8	0	0
7	407	2	730	6	7	1
10	1,815	2	42,917	5	0	0
13	268	2	36,595	8	25	1
16	450	2	290	4	15	1
20	160	1	2,478	8	0	0
24	488	2	14,298	7	0	0
29	56	1	139	4	0	0
31	167	2	1,447	8	0	0
36	160	1	9,605	5	0	0
41	160	1	296	4	0	0

This variability in the numbers of the flagellates is illustrated in Table III, where are given the number of *Heteromita globosa* found in the first 12 samples.

Table III
Heteromita globosa, numbers per gram. of soil

Sample	Soil extract	Peptone
1	10,400	1,800
2	1,800	1,800
3	3,600	160
4	640	480
5	450	230
6	56	32
7	640	230
8	320	6.8
9	79	0
10	3,600	3,600
11	5,200	640
12	450	0

It is seen that there is no consistent difference between the numbers from the two agars; in some samples they are alike or only show slight differences and, in others, the differences are more than twentyfold.

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Samples 6, 9 and 12 with low numbers of *H. globosa* are all deep samples taken from a depth of 50–55 cm.

Table IV shows the numbers of *Hartmanella hyalina* on the two agars.

Table IV
Hartmanella hyalina, numbers per gram of soil

Sample	Soil extract	Peptone
1	59,000	10,400
2	7,200	450
3	5,200	25
4	640	450
5	320	15
6	56	6.8
7	900	450
8	160	6.8
9	900	6.8
10	20,800	1,800
11	5,200	900
12	20,800	6.8

These figures demonstrate that peptone agar gives a very low estimate of the numbers of *H. hyalina* and, though these are always larger in soil extract agar, there is still a great variability in the increase.

Appearance of the agars

The appearance of the two sets of Petri dishes is quite distinct. Peptone agar encourages the growth of specialized species of bacteria; while soil extract agar allows of the development of many species in small numbers. On peptone agar plates large numbers of *Cercomonas crassicauda*, *Heteromita globosa* and *Oicomonas termo* occur which swamp other organisms, sometimes making it very difficult to identify other species in plates of low dilutions. On soil extract agar the Protozoa though fewer in numbers on the individual plates are of varied species, and no individual species swamp the development of other forms. All types of Protozoa flourish on this agar, the growth of amoebae and ciliates is most marked, especially that of the latter which are never very abundant in the soil and are usually not recorded when peptone agar is used. The soil extract, as seen in Table II, supports a greater diversity of species of Protozoa as well as higher numbers.

The following list shows the Protozoa recorded from Russian soils on the two media. The symbols S.E.A. represent soil extract agar and P.A. peptone agar.

*The Protozoa recorded from Russian soils on soil extract
agar and peptone agar*

FLAGELLATA.

- Cercomonas crassicauda* Alexeief, S.E.A., P.A.
C. longicauda Stein, S.E.A., P.A.
Cercobodo agilis Moroff, S.E.A., P.A.
Helkesimastix faecicola Woodcock, S.E.A., P.A.
Phalansterium solitarium Sandon, S.E.A., P.A.
Bodo celer Klebs, S.E.A.
B. saltans Ehrbg., S.E.A., P.A.
Heteromita globosa Stein, S.E.A., P.A.
Spiromonas angusta Duj., S.E.A.
Sainouron mikroteron Sandon, S.E.A., P.A.
Allantion tachyploon Sandon, S.E.A., P.A.
Tetramitus rostratus Perty, S.E.A.
T. spiralis Goodey, S.E.A., P.A.
Oikomonas termo (Ehrbg.) Martin, S.E.A., P.A.
O. mutabilis Kent, S.E.A., P.A.
Scytomonas pusilla Stein, S.E.A.
Entosiphon sulcatum (Duj.) Stein, S.E.A., P.A.
Allas diplophysa Sandon, S.E.A.

RHIZOPODA NUDA.

- Naegleria gruberi* (Schardinger) Wilson, S.E.A., P.A.
Hartmanella hyalina (Dangeard) Alex, S.E.A., P.A.
Amoeba diploidea Hartmann & Nagler, S.E.A., P.A.
A. striata Penard, S.E.A., P.A.
A. albida Nagler, S.E.A.
A. radiosa type, Ehrbg., S.E.A.
A. glebae, Dobell, S.E.A.
Amoeba sp. α, Sandon, S.E.A., P.A.
Biomyza vagans Leidy, S.E.A.
Gephyramoeba delicatula Goodey, S.E.A.
Nuclearia simplex Cienk, S.E.A.
Actinophrys sol Ehrbg., S.E.A.
Acanthocystis aculeata Hertwig & Lesser, S.E.A., P.A.

RHIZOPODA TESTACEA.

- Euglypha tuberculata* Duj., S.E.A., P.A.
Trinema lineare Penard, S.E.A.
Lecythium hyalinum (Ehrbg.) Hertwig & Lesser, S.E.A., P.A.
L. mutabile Bailey (Hopk.)

CILIATA.

- Enchelys farcimen* Ehrbg., S.E.A.
Chilodon uncinatus Ehrbg., S.E.A.
Colpoda cucullus O.F.M., S.E.A.
C. steinii Maupas, S.E.A., P.A.
Cinetochilum margaritaceum Ehrbg., S.E.A.
Balantiophorus elongatus Schew., S.E.A.
Blepharisma lateritia var. *minima* (Ehrbg.) Roux, S.E.A.
Lembus pusilla (Quenn) Calkins, S.E.A.
Halteria grandinella O.F.M., S.E.A.
Uroleptus musculus Ehrbg., S.E.A.
U. mobilis Englm., S.E.A., P.A.
Gastrostyla steinii Englm., S.E.A.
Pleurotricha lanceolata Ehrbg., S.E.A.
Vorticella microstoma Ehrbg., S.E.A.

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The list of species from peptone agar gives the impression of a greater number than actually occurs in any one sample, as the only flagellates constantly found were *Cercomonas crassicauda*, *Heteromita globosa*, *Sainouron mikroteron* and *Oicomonas termo*; the other species occurring only occasionally. The same thing is found with the Rhizopoda, *Hartmanella* being the only constant species; and of the ciliates, *Colpoda steinii* was very occasional and *Uroleptus mobilis* very rare in peptone agar, though in soil extract agar ciliates were usually recorded, but never in large numbers.

Distribution of the Protozoa

As the Russian soils were of so many types it was hoped to find some correlation between the type of soil and the protozoan numbers or species present. The investigations indicate that the protozoan fauna is very similar in all soils. The highest numbers found in any of these soils is in sample 13 described as a light grey forest steppe soil; this sample was taken to a depth of 10 cm. and gave a total of 555,183 Protozoa per gram. Table V shows the numbers of flagellates, ciliates and amoebae

Table V
Numbers of Protozoa in Russian soils (surface samples)

No. of sample	Amoebae	No. of species	Flagellates	No. of species	Ciliates	No. of species
Chernozem						
1	59,268	8	187,874	10	118	4
2	7,374	4	64,263	9	253	4
3	4,454	4	262,762	4	0	0
49	7,363	7	8,130	10	161	3
Degraded chernozem						
10	21,250	2	8,402	8	175	2
Light grey forest steppe						
13	121,938	6	433,159	8	86	2
Humus carbonate						
41	1,322	3	75,254	7	38	1
Podsolized soil						
4	960	3	2,783	10	22	2
16	1,901	4	737	8	15	1
20	3,710	2	3,656	8	32	2
24	3,625	5	45,017	9	40	2
29	117	2	2,597	5	0	0
36	3,620	4	10,010	8	38	1
52	3,670	4	44,576	10	60	3
54	5,720	5	32,913	10	117	2
Red brown heavy forest clay						
7	1,321	7	2,746	12	117	2
Alluvial						
31	2,600	1	277,185	9	7	1

that make up this number. Light grey forest steppe soil is derived from the chernozems which, when exposed to great leaching, become degraded chernozem and pass by a further degradation into light grey forest soil. If the light grey forest steppe soil is classified with the chernozems, there is a distinct indication that higher numbers of Protozoa are found in the chernozem soils. Full descriptions of these soils are given by Robinson (8).

DEPTH SAMPLES

The numbers and species of Protozoa failed to show any definite relation with the soil depths, though, as a general rule, more species and numbers of individuals were found in the top layers. The duplicate sets on peptone and soil extract agars usually gave similar relative numbers, fewer developing on the peptone agar.

Flagellata

Flagellates are the most abundant in number and species, 25 species being recorded in all; some were present in every soil (see Table VI). The common flagellates of the 55 soils examined are *Heteromita globosus* found in 98 per cent of the soils, *Cercomonas crassicauda* and *Oikomonas termo* in 96 per cent, and *Bodo saltans* in 89 per cent. The occurrence of *B. saltans* is interesting as Sandon (9) records this as not at all common in soils, but it was found by him in a Mauritius soil and by Nowikoff (6) in a Russian soil. *Sainouron mikroteron* was found in 83.6 per cent, *Allantion tachyploon* in 80 per cent. The following six species, *Heteromita globosa*, *Cercomonas crassicauda*, *Oikomonas termo*, *Bodo saltans*, *Sainouron mikroteron* and *Allantion tachyploon* can be regarded as the commonest species in the soils examined.

Table VI

Mastigophora. Number of occurrences in percentages in Russian soils

<i>Cercomonas crassicauda</i> Alexeieff	96	<i>Sainouron mikroteron</i> Sandon	83
<i>C. longicaudus</i> Stein	29	<i>Allantion tachyploon</i> Sandon	80
<i>Cercobodo agilis</i> Moroff	56	<i>Spongomonas</i> sp., Stein	5
<i>C. vibrans</i> Sandon	1	<i>Tetramitus rostratus</i> Perty	3
<i>Helkesimastix faecicola</i> Woodcock & Lapage	61	<i>T. spiralis</i> Goodey	41
<i>Phalansterium solitarium</i> Sandon	60	<i>Oikomonas termo</i> Ehrbg.	96
<i>Bodo edax</i> Klebs	1	<i>O. mutabilis</i> Kent	21
<i>B. saltans</i> Ehrbg.	89	<i>Srytomonas pusilla</i> Stein	21
<i>B. celer</i> Klebs	1	<i>Astasia proteus</i> Ehrbg.	1
<i>Heteromita globosa</i> Stein	98	<i>Anisonema minus</i> Sandon	3
<i>H. ovata</i> Duj.	1	<i>Entosiphon sulcatum</i> (Duj.) Stein	32
<i>Spiromonas angusta</i> Duj.	18	<i>Allas diplophysa</i> Sandon	25

Wassilewsky (11) in her work on flagellates from Russian soils examined soils from 18 different localities. She records no more than four species

of flagellates in any soil. One sample was from Catherinander (Kuban) now named Krasnodar. The three samples in this present investigation from that region, gave respectively ten, nine and four flagellates, while Wassilewsky records only *Monas termo* which she finds in soils of 13 regions. This is probably *Oikomonas termo*. Her other commonest flagellate was *Amphymonas globosa* as *Heteromita* was not recorded; this is probably *Heteromita globosa*. *Cercomonas crassicauda* and *C. longicaudus* were also found. Yakimoff & Zeren (12) did not record *Heteromita globosa*. Sandon (9) suggests that *Prowazekia ninae-kohlyakimov*, which is recorded as frequent, must be this species. *Oikomonas termo* is probably also recorded by them either as *Monas termo* or included in *Oikomonas mutabilis*, and referred to as frequent. *Cercomonas crassicauda* is, however, one of the dominant species found by Yakimoff & Zeren (12).

The total number of flagellates found in the tobacco soils is shown in Table VI, with the percentage occurrence in the soils. Considering that most of the soils examined were unmanured, the number of flagellates, both species and individuals, found in the upper layers of the soils is unusually high and varied. With a few exceptions the greatest number of flagellates are found in the upper layers of the soil.

Ciliata

Ciliates were not abundant in any of the soils, though 25 species were found; three only occurred in any abundance. As none was common in the peptone agar plates, these statements apply to the Ciliata recorded from the soil extract, soil extract agar, hay infusion and distilled water. The three most common species are *Colpoda steinii*, *C. cucullus* and *Balantiophorus elongatus*. With the exception of *Cinetochilum margaritaceum*, they were all the usual soil types, such as occur more or less commonly in the Rothamsted soils. Table VII shows the percentage occurrences of the ciliates recorded.

Table VII

Ciliata. Number of occurrences in percentages in Russian soils

<i>Holophyra</i> sp., Ehrbg.	3	<i>Balantiophorus minutus</i> Schew.	14
<i>Urotricha farcta</i> Clap. & Lach.	3	<i>Lembus pusilla</i> (Quenn) Calkins	20
<i>Enchelys farcimen</i> Ehrbg.	7	<i>Blepharisma</i> sp., Perty	12
<i>Glaucoma scintillans</i> Ehrbg.	3	<i>Halteria grandinella</i> O.F.M.	3
<i>Dileptus gigas</i> Duj.	2	<i>Uroleptus musculus</i> Ehrbg.	12
<i>Chilodon uncinatus</i> Ehrbg.	25	<i>U. mobilis</i> Englm.	9
<i>Colpoda cucullus</i> O.F.M.	50	<i>Gastrostyla steinii</i> Englm.	12
<i>C. steinii</i> Maupas	69	<i>Gonostomum affine</i> Stein	5
<i>C. maupasii</i> Enriques	3	<i>Pleurotricha lanceolata</i> Ehrbg.	3
<i>Pleuronema chrysalis</i> Ehrbg.	2	<i>Ozytricha platysoma</i> Ehrbg.	5
<i>Cinetochilum margaritaceum</i> Ehrbg.	7	<i>Euplotes patella</i> Ehrbg.	2
<i>Balantiophorus elongatus</i> Schew.	32	<i>Vorticella microstoma</i> Ehrbg.	3

Dileptus gigas was an unusual species only found in one sample, a humus carbonate soil. This has been recorded by Yakimoff & Zeren (12) who call it rare, and by Losin Losinsky (7) from heavy steppe soil.

Halteria grandinella is another ciliate recorded by Yakimoff & Zeren (12) as rare. This was probably found by Nowikoff (6) and Losin Losinsky who both record *Halteria* sp.

Glaucoma scintillans, which was found in two soils from Krasnodar, has also been recorded in one soil by Yakimoff & Zeren (12). *Cinetochilum margaritaceum*, which was found in five soils, appears to be unrecorded as a soil type. *Cinetochilum* was never common, being in insufficient numbers to be recorded in the dilution counts; it developed in soil extract, sterile water and hay infusion.

Rhizopoda

Twenty-five species of Rhizopoda were found in all, but of these only three were common; Rhizopoda testacea were very poorly represented.

Hartmanella hyalina is the typical common species found in 98 per cent of the soils; this amoeba is recorded by Sandon (9) as being very widely spread in most localities that have been investigated.

A very small *Limax* amoeba, referred to by Sandon as species α and found by him in 13 soils, has frequently been found in the Russian soils. Sandon failed to isolate this form in pure culture; it is not certain if this is a true species or a small *Limax* stage in the life history of more than one amoeba.

A *radiosa* type of amoeba occurs in many soils but, as a number of different species assume this form, it is impossible to identify these species. *Amoeba diploidea*, which is found in 49 per cent of the soils, is a characteristic soil type and has been found in a wide range of soils. The chief types of characteristic amoebae found in these soils were *Hartmanella hyalina*, *Amoeba* sp. α , *A. diploidea* and *A. radiosa* type. The total number of Rhizopoda recorded is shown in Table VIII. Two species only were found belonging to the Reticulosa family; these were *Biomyxa vagans* and *Gephyramoeba delicatula*. Both of these organisms were rare but, as both can easily be overlooked, they and other species of the same family may be more common than these records indicate. *Nuclearia simplex* was only found in 25 soils, though this is a usual soil type, having been found frequently by Sandon (9); it does not appear to have been recorded for Russian soils before. Two species of Heliozoa were recorded, *Actinophrys sol* and *Acanthocystis aculeata*, the former

having been reported from Russia by Yakimoff & Zeren⁽¹²⁾ in two soils.

Testaceous Rhizopoda were not common in any of the soils. The three most common were *Lecythium hyalina*, *Euglypha tuberculata* and *Trinema enchelys*, which are typical soil forms and have been recorded from various types of soil. As is seen from Table VIII, other Testaceous Rhizopoda were found occasionally, but this group was very poorly represented. This may be due to their slow development in cultures; these were examined at intervals during a period of 28 days, instead of the usual period of 14 days, in order to include the late developing species.

Table VIII

Rhizopoda. Number of occurrences in percentages in Russian soils

RHIZOPODA NUDA.

<i>Naegleria gruberi</i> (Schardinger) Wilson	20
<i>Hartmanella hyalina</i> (Dangeard) Alex.	98
<i>Amoeba</i> sp. α, Sandon	65
<i>A. diploidea</i> Hartmann & Nagler	49
<i>A. striata</i> Penard	16
<i>A. albida</i> Nagler	7
<i>A. glebae</i> Dobell	5
<i>A. horticola</i> Nagler	1
<i>A. actinophora</i> Auerbach	1
<i>A. radiosa</i> type Ehrbg.	40
<i>Biomyxa vagans</i> Leidy	7
<i>Gephyramoeba delicatula</i> Goodey	3
<i>Nuclearia simplex</i> Cienk.	36
<i>Actinophrys sol</i> Ehrbg.	10
<i>Acanthocystis oculata</i> Hertwig & Lesser	1

RHIZOPODA TESTACEA.

<i>Arcella vulgaris</i> Ehrbg.	1
<i>Diffugia constricta</i> (Ehrbg.) Leidy	3
<i>Euglypha tuberculata</i> Duj.	32
<i>Trinema enchelys</i> Ehrbg. (Leidy)	18
<i>Lecythium hyalina</i> (Ehrbg.) Hertwig & Lesser	32
<i>L. mutabile</i> (Bailey) Hopk.	3

Summary

The protozoan population of these tobacco soils were typical species generally found in soils. No soils were free of Protozoa, though very few were found in some of the lower depth samples and in the sandy soils.

Though there was an indication that higher numbers of Protozoa were found in the chernozem soils, no definite connexion was observed between either the numbers of Protozoa or the species with the types of soil examined.

The numbers generally showed a decrease with increasing depth of soil; there were a few exceptions to this.

PART II. ENGLISH SOILS

In view of the striking difference in the growth of the Protozoa on soil extract agar compared with that on peptone agar, four English soils were examined in the same manner as the Russian soils.

The soils chosen were from the farmyard manured and unmanured plots of Barnfield, a very heavy clay soil with flints; and from the unmanured and previously farmyard manured plot from Stackyard Continuous Wheat Field at Woburn Experimental Station. The latter had been treated with farmyard manure for a period of 50 years until 1925 and had been seven seasons without manure when the samples were taken. The results are shown in Table IX.

Table IX

	Soil extract agar		Peptone agar	
	Total no.	No. of species	Total no.	No. of species
Barnfield farmyard manured	74,494	22	23,392	13
Barnfield unmanured	19,221	13	3,369	6
Woburn farmyard manured	8,056	20	1,360	9
Woburn unmanured	371,032	12	1,111	6

A further experiment was carried out to test whether the peptone agar could be replaced by a liquid medium, the soil used being from the farmyard manured plot of Barnfield, Rothamsted. The media were soil extract made from the plot soil, hay infusion and two peptone agars. In one of the two agars, steaming replaced the usual autoclaving, and the sterilizing was effected by steaming the agar on three successive days. Platings were made from the same set of dilution bottles on to each of the four media so that the variation in species and numbers can only be due to the effect of the media on the population. The cultures were examined after 7 and again after 14 days.

The results of this experiment are shown in Table X; the soil extract and hay infusion give a greatly increased count for the amoebae and ciliates as was the case with the soil extract agar. A total of 30 species was recorded from soil extract, compared with 20 from hay infusion, 10 from ordinary peptone agar and 15 from steamed peptone agar.

Liquid soil extract, like soil extract agar, encourages the growth of many species, but of few individuals, and the scarcity of the individuals in the liquid media makes this more difficult to examine than agar media. Scraping the surface of the agar usually gives a representative sample of Protozoa, but, in a liquid culture, two or three loopfuls of the media

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does not always do this as some species, particularly amoebae, may adhere to the bottom of the culture and some free swimming ciliates remain on the bottom particles.

Table X

*Total number of Protozoa per gram of soil in Barnfield
farmyard manured plot*

Sample	Soil extract		Hay infusion		Agar autoclaved		Agar steamed	
	Total no.	Species	Total no.	Species	Total no.	Species	Total no.	Species
Flagellata								
1	129,393	8	26,577	9	41,877	5	—	—
2	69,781	9	47,221	7	105,493	6	120,967	5
3	317,291	8	242,046	8	382,838	4	473,160	4
4	102,116	7	59,189	7	128,529	7	—	—
Amoebae								
1	22,387	7	1,081	5	148	2	—	—
2	3,942	5	1,035	3	506	2	169	5
3	14,414	3	3,814	4	575	3	1,009	4
4	2,614	3	912	3	450	1	—	—
Ciliata								
1	125	2	94	2	0	0	—	—
2	194	6	106	4	15	1	7	1
3	363	6	573	7	15	1	28	4
4	257	8	57	3	0	0	—	—

The results in the case of flagellates are not clear. Eleven species were found on soil extract, compared with ten on hay infusion, seven on ordinary peptone agar and five on steamed peptone agar. Though the number of flagellate species was always greater in the soil extract, in three samples there was a great development of the three common species, *Cercomonas crassicauda*, *Heteromita globosa* and *Oikomonas termo*, which raised the numbers on peptone agar, as can be seen in Table X.

Peptone agar which was steamed instead of autoclaved was used with two samples of soil; on these it gave a greater number of amoebae and ciliates and fewer species of flagellates, though the actual numbers counted were higher than with ordinary peptone agar. The following list shows the species which developed on the four media, and illustrates the poor development of amoebae and ciliates on peptone agar. The symbols S.E. represent soil extract, H.I. hay infusion, P.A. 1 peptone agar autoclaved, P.A. 2 peptone agar steamed. The nomenclature of the Protozoa is according to Sandon (9).

*Protozoa recorded from Barnfield farmyard manured plot
on different media*

FLAGELLATA.

- Cercomonas crassicauda* Alexeiff, S.E., H.I., P.A. 1, P.A. 2
Cercobodo agilis Moroff, S.E.
Mastigamoeba limax Moroff, S.E.
Bodo saltans Ehrbg., S.E., H.I.
Helkesimastix faecicola Woodcock & Lapage, H.I.
Heteromita globosa Stein, S.E., H.I., P.A. 1, P.A. 2
Spiromonas angusta Duj., S.E., H.I.
Sainouron mikroteron Sandon, S.E., H.I., P.A. 1, P.A. 2
Allantion tachyploon Sandon, S.E., H.I., P.A. 1, P.A. 2
Spongomonas sp., Stein, S.E., H.I., P.A. 1
Tetramitus spiralis Goodey, S.E., H.I., P.A. 1
Oikomonas termo (Ehrbg.) Martin, S.E., H.I., P.A. 1, P.A. 2

RHIZOPODA NUDA.

- Naegleria gruberi* Schardinger (Wilson), P.A. 2
Hartmanella hyalina (Dangeard) Alex, S.E., H.I., P.A. 1, P.A. 2
Amoeba diploidea Hartmann & Nagler, S.E., P.A. 1, P.A. 2
A. radiosa type, Ehrbg., S.E.
Amoeba sp. α, Sandon, S.E., H.I., P.A. 2
Biomyxa vagans Leidy, S.E.
Nuclearia simplex Cienk., S.E.
Acanthocystis aculeata Hertwig & Lesser, S.E.

RHIZOPODA TESTACEA.

- Diffugia globula* Ehrbg., S.E.
Euglypha tuberculata Duj., S.E.
Lecythium hyalinum (Ehrbg.) Hertwig & Lesser, S.E., H.I., P.A. 2

CILIATA.

- Spathidium spathula* O.F.M., S.E., H.I., P.A. 2
Chilodon uncinatus Ehrbg., S.E.
Colpoda steinii Maupas, S.E., H.I., P.A. 1, P.A. 2
C. cucullus O.F.M., S.E., H.I.
Pleuronema chrysalis Ehrbg., S.E.
Balantiophoris elongatus Schew., S.E., H.I.
Halteria grandinella O.F.M., S.E.
Uroleptus musculus Ehrbg., S.E., H.I., P.A. 2
Gastrostyla steinii Englm., S.E., H.I., P.A. 2
Vorticella microstoma Ehrbg., S.E., H.I.

These experiments show that a peptone medium gives a low estimate of Protozoa present in the soil, this being true for all classes, flagellates, ciliates and amoebae. When soil extract is used in the place of peptone agar, the increase in the number of species and individuals is striking with ciliates and amoebae. Soil extract and hay infusion give a higher development of species than peptone agar, but are not as good for the development of the common types of soil flagellates.

Table X shows that except with flagellates the numbers of Protozoa are highest on soil extract, which always encourages a large number of species of all types.

CONCLUSIONS

These results of this investigation show that nutrient peptone agar is unsatisfactory as a medium for counting the numbers of soil Protozoa. Soil extract agar and liquid soil extract give a more representative record of all classes of Protozoa, but particularly of the amoebae and ciliates present in the soil. The numbers of flagellates recorded on soil extract are sometimes less than when peptone agar is used as a medium, even though more species of flagellates develop. Hay infusion, which was also used, gives a far greater number of species, particularly ciliates, than does peptone agar, but the numbers recorded are less than with soil extract or soil extract agar.

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and acetic acids can be oxidised on a percolating filter quite as readily as, if not more rapidly than, sucrose solutions of equal concentration and biochemical oxygen demand. Proteolysis is chiefly an anaerobic fermentation and of little if any importance in biological purification, which is essentially aerobic. It is generally confined to sludge digestion and plays only a minor part in the purification of settled effluents. The admission of effluents containing lactic and similar acids to municipal sewage plants would probably only cause difficulty provided the biochemical oxygen demand of the crude liquor was too great for the capacity of the filters used. The success of Levine's preliminary filtration was probably due largely to the reduction of the biochemical oxygen demand; in other words the preliminary filters reduced the load on the main sewage filtration plant.

The use of lime for neutralising the acid appears to be unnecessary since the free acid is very readily oxidised without the troublesome precipitation of calcium carbonate produced by the oxidation of the lactate. The cost of liming and the handling of the increased weight of sludge has no compensating advantages in increased rate of purification and its use appears to be based on a confusion of sludge digestion with biological purification.

Eldridge⁸ obtained satisfactory purification on a gravel filter of milk wastes having a B.O.D. up to 160 parts per 100,000 and found that stronger liquids were liable to clog the filter. He recommended the use of a storage tank with a 4-hour detention period.

In the following pages experiments are described showing the limitations of direct filtration and the advantage of a pretreatment in a septic tank. The changes occurring in milk effluents during storage in a septic tank are set out and it is shown that the effect of changes of p_H on proteolysis in the absence of added sugar are normally of little importance in a storage tank when septic conditions are well established. Subsequent treatment of the tank effluent on a percolating filter gives very satisfactory results and it is concluded that the tank effluent after storage for 24 hours can be suitable for admission in limited amounts to municipal sewage plants.

Direct treatment on a percolating filter

The apparatus used in this experiment was the same battery of sectional filters previously used in experiments on the biological filtration of dilute sucrose solutions¹ (see Fig. 1). The filter medium was washed gravel passing 1-in. mesh but retained by $\frac{1}{2}$ -in. mesh.

Preliminary tests of the biochemical oxygen demand of milk solution showed that a 1.3% solution of fresh milk has about the same oxygen demand as the 0.2% sucrose used in the previous experiment, viz., 130 parts per 100,000. A 1.3% solution of fresh milk was therefore used in these experiments and the rate of flow was 100 gals. per cu. yard per day. Since washing soda is used as a cleaning agent in dairies and milk factories, the effect of this alkali on the rate of filtration was

investigated by running four sets of filters with additions of soda sufficient to raise the p_H of the mixtures containing 1.3% of fresh milk from 7 to 8, 9, and 10 respectively. A consideration of the analysis of fresh milk shows that the C/N and C/P₂O₅ ratios are adequate for the nutritional requirements of micro-organisms, so that no additions of either nitrogen or phosphates were considered necessary as was the case with sugar solutions. No special inoculation was given to the filter medium, which had been cleaned by agitation with tap-water.

The filters were run for a period of one month and tests were made at intervals of several days during this period. The results of these tests are given in Table I.

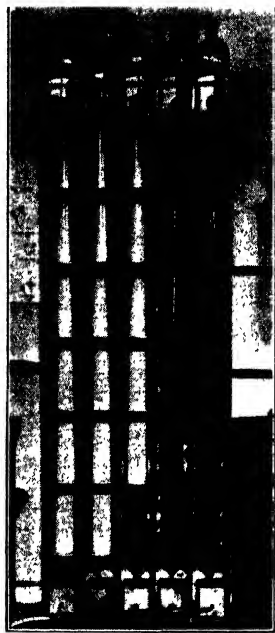


Fig. 1

It will be seen that the development of the filters was rapid; over 90% purification over all six sections was reached in 5 days with the mixtures of initial p_H values of 7 and 8 and over 80% with mixtures of initial p_H values of 9 and 10. It should be noted that the temperatures were those normal in the laboratory during summer months. The effect of the soda appeared to be to delay the growth of the biological film in the first sections, but it had little or no effect on the lower sections. During its passage through the first section the p_H of the liquid supplied to filter *D* dropped from 10 to 8, probably owing to the production of carbon dioxide and formation of sodium bicarbonate. After three weeks there was a fall in the amount of purification achieved by the filters; this appeared to be due to excessive accumulation of film in the first two sections (see previous paper¹).

⁸ Michigan Eng. Exp. Sta., Bull. 28, 1930; Eng. News-Rec., 1931, 106, 520.

Filters *A* and *B* were obviously becoming choked during the fourth week and *C* and *D* showed signs of developing a similar condition.

TABLE I

Treatment of mixtures containing 1.3% of milk in percolating filters arranged in six sections. Percentage purification as measured by test for biochemical oxygen demand

Period, days	Section of filter	<i>A</i> p_H 7.0	<i>B</i> p_H 8.0	<i>C</i> p_H 9.0	<i>D</i> p_H 10.0
5	6	97	97	88	82
7	6	96	97	96	91
10	1	62	58	52	47
	2	88	72	64	59
	3	93	89	86	70
	4	95	94	90	88
14	1	58	56	56	58
	2	90	92	89	88
	3	96	98	93	96
21	1	35	40	43	37
	2	87	90	92	92
	3	96	98	97	96
28	3	86	87	82	93

At the end of the fourth week the film on the filtering medium was examined; it consisted of a dense white mass of fungal hyphae and actinomycetes in which were embedded masses of fat globules. The dry matter of the film contained 5% of total nitrogen and extraction with light petroleum gave a yield of 65% of fat on the dry matter of the film in the top sections of filters *A* and *B*. This large proportion of fat probably resulted from the breaking of the emulsion of the milk and mechanical straining out of the fat globules during the passage of the liquid through the fungal mycelium. The apparent resistance of the fat to biological oxidation is probably due to its occurrence in dense aggregates not so readily available for bacterial nutrition as it would be were it maintained in a state of emulsion.

The top section of one of the filters was then replaced by a section constructed of wooden laths arranged in the same manner as in filters previously designed and employed by Levine as mentioned above.

The laths were fixed in the form of wooden grids placed one above the other with the direction of the strips of one grid at right angles to that of the strips in the grids immediately above and below; the grids were each 6 in. square and were stacked to form a section 10 in. in height (see Fig. 2). After a preliminary seeding for three days with an inoculated mixture con-

taining milk the filter was supplied with a mixture containing 1.3% of fresh milk at 100 gallons per cu. yard per day as before.

Growth of the film through the grids was easily visible and in two weeks it formed a continuous vertical column about 2 in. in diameter through the whole of the wooden section. At the end of the third week the film occupied more than half the area of the grids. The results during this period are given in Table II.

The results are similar to those previously obtained with gravel alone. The film in the top section was similar in texture and composition to the film from the top section of gravel. Owing to the more open arrange-

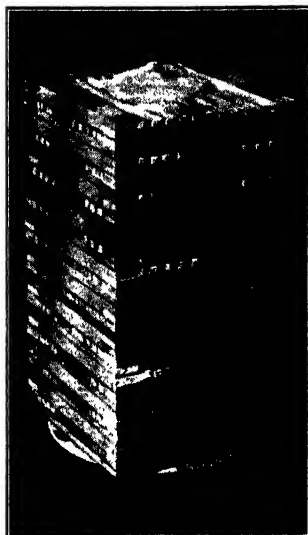


FIG. 2

ment, however, the section of wooden laths would not be choked so rapidly as gravel and could be more readily removed and cleaned.

Septic tank treatment

The septic tank used in these experiments consisted of a rectangular glass tank of 15 litres capacity. When filled to a depth of 9 in. it held 12 litres of liquid and 120 ml. of milk constituted a charge. In the first experiment no special inoculation was used, and the growth of organisms was left entirely to chance. The duration of treatment in the tank was varied and the fermentative changes occurring during these periods were measured in terms of p_H , biochemical oxygen demand in 5 days, and formol titration value for amino-acids. The numbers of bacteria were also counted by the hæmocytometer. By means of a siphon 10 litres of liquor could be withdrawn as desired from between the bottom sludge and the top scum without disturbing either. On recharging, the whole contents of the tank were thoroughly agitated. The results obtained during the first three months are given in Table III.

TABLE II

Treatment of mixture containing 1.3% of milk in percolating filter with a top section of wooden laths, the lower section containing gravel

Period, days	Section	Purification, %
3	1 (wooden lath)	45
	2 gravel	62
	3 "	77
6	1	57
	2	69
	3	86
4	1	60
	2	81
	3	95

also prevented any precipitation of the milk protein (nos. 7 and 8). It follows from these results that the precipitation of milk proteins in a septic tank depends on the development of sufficient acidity to reach a p_H near 4.6 before the proteolytic action of the bacteria has converted the casein into the more soluble proteoses and amino-acids. Lactic and proteolytic fermentations are mutually antagonistic. Absence of aeration favours lactic fermentation, which in a mixture of low buffer capacity results in a rapid fall in p_H to a value about 4, thus inhibiting the growth of the proteolytic bacteria. This occurred in treatment 3 using distilled water; casein was completely precipitated, giving a clear liquid which remained clear indefinitely at p_H 4.2 and free from putrefactive odours. When Harpenden tap water was used, its natural buffer capacity delayed the reduction in p_H , which did not fall below 5.5 before proteolysis began, resulting in reduction of acidity, increased solubility of the protein, and the development of putrefactive odours. Using a septic tank liquor with a richer proteolytic flora the precipitation of the casein may be entirely prevented. It follows from this that the nature of the water used for dilution may determine the nitrogen content of the sludge formed in septic tank treatment and the nitrogen content of the solution to be treated by subsequent filtration.

The presence of solid curd in waste waters and washings from milk factories would increase the nitrogen content of the sludge, unless alkali or soap is used in the wash waters, which would promote dissolution of the casein and its subsequent hydrolysis.

In the experimental septic tank using tap water the p_H of the liquid never fell below 5.5, and the addition of 0.2% of hydrochloric acid was required to reduce this figure to 4.6 (Table VI). This caused a drop in bacterial numbers from 500 to 180 millions per ml. Addition of 0.1% of sodium carbonate maintained the p_H above 7.0 without any significant effect on the bacterial numbers, but appeared to have increased their proteolytic action as measured by the formol titration value for amino-nitrogen. Typical figures for bacterial numbers, acidity, and amino-nitrogen are given in Table VI.

TABLE VI

Characteristics of septic tank liquors from mixtures containing 1% of milk

	p_H	Bacterial numbers, millions per ml.	Formol titration, ml. 0.05N-soda	B.O.D., parts per 100,000
Normal	5.6	500	1.8	80
+0.2% HCl	4.6	180	0.5	45
+0.1% Na_2CO_3	7.4	550	2.5	80

Subsequent analysis of the sludge from the alkaline mixture showed that it contained less than 0.4% of nitrogen and all the fat was present as insoluble soap. The effect of septic actions on waste washing waters from dairies will depend chiefly on the following three factors: (1) the concentration of dissolved bases originally in the wash water used; (2) the concentration of alkali added during washing; (3) the concentration of sugar in the effluent.

In the effluents from factories engaged chiefly in the bottling and distribution of fresh milk, the concentration of alkali may be comparatively high and there may be considerable proteolysis during septic action. In factories producing sweetened condensed milk and in those making cheese the effluents will be rich in sugar and the washings from cheese vats will be poor in protein, in which case acid fermentation would predominate in the septic tank and there would be little or no proteolysis.

Biological filtration of effluents from septic tanks

Tank liquors obtained in the preceding experiments were treated at the rate of 100 gals. per cu. yard per day in laboratory sectional filters containing gravel graded $\frac{1}{2}$ in. to $\frac{1}{4}$ in. The results obtained during a period of several months are given in Table VII.

TABLE VII

Purification of septic tank effluent on percolating filter

Period, weeks	Effluent	B.O.D., parts per 100,000	Purification, %
1	Tank	62	—
	Section 3	52	15
	" 6	36	42
2	Tank	60	—
	Section 3	45	25
	" 6	10	83
3	Tank	68	—
	Section 3	44	35
	" 6	10	85
4	Tank	72	—
	Section 3	28	61
	" 6	1.4	97
5	Tank	74	—
	Section 3	3.0	60
	" 6	1.5	98
16	Alkaline effluent, p_H 8.0	74	—
	Tank	25	68
	Section 3 ...	1.6	98
17	Tank	84	—
	Section 3	3.1	63
	" 6	1.7	98

Development of biological film on the filtering medium appeared to be somewhat slower than with untreated mixtures containing 1% of fresh milk and the effluent was not effectively purified until the end of the fourth week. Analysis of the film after 20 weeks showed a complete absence of fat and the filter showed no signs of ponding. During frosty weather when the temperature of the tank fell to 4° C., the biochemical oxygen demand of the liquid rose to 120 with a corresponding increase in the B.O.D. of the filter effluents. At such times the tank liquor contained most of the lactose unchanged and little if any separation of solids occurred in the tank. Such conditions would be unlikely on the large scale with a tank in continuous operation. Analyses of the effluent showed ammonia equivalent to 3.5 parts of nitrogen per million and nitrate equivalent to 10.5 parts of nitrogen after three weeks. At this period the loss of nitrogen during filtration reached 14 parts out of a total of 44 parts in the crude liquor. When the filter had been in operation for 3 months the production

f nitrate-nitrogen reached a maximum of 56 parts per million, which is more than the nitrogen contained in the crude liquor; this indicates a breakdown of the protein-nitrogen retained in the filter during the early stages.

Some samples of the final effluent showed a dissolved oxygen content of 0.53 part per 100,000 and when incubated without dilution remained quite odourless for several weeks, indicating a stable effluent.

DISCUSSION

Mixtures containing 1% of fresh milk in water possess the organic and inorganic substances required for bacterial growth and in this respect are suitable for treatment by biological oxidation in percolating filters. Mechanical separation of the fat, however, with its accumulation in the filter, inhibits its own oxidation and causes clogging of the filter, resulting in a low rate of purification. This effect appears to be associated with an abnormal growth of fungi.

By preliminary fermentation in a septic tank a complete separation of the fat can be obtained. At temperatures below 10° C. this fermentation may be delayed so that a long period is necessary for complete separation of the fat to occur. This accounts for stronger tank liquors being produced in winter than in summer. The amount of protein which separates out in this process is dependent on the changes in reaction (p_H) during fermentation. Lactose or a mixture containing 1% of milk is about 0.05% and is sufficient to reduce the p_H to about 4.0 provided the water is free from bases, but the use of hard water or of soap or soda in the wash waters prevents the development of high acidities and little separation of protein then occurs. Even under conditions of high acidity and complete precipitation of casein, the separated liquid still contains 0.0025% of soluble nitrogen, which is sufficient to give a C/N ratio with the lactose (or lactic acid 0.05%) of 8. Treatment of a liquid of this composition by biological oxidation in percolating filters should be possible without difficulty.¹

In the case of milk factories using sugar, an increase in the sugar content of the effluents would not only give rise to high acidities in the tank and consequent reduction of the nitrogen content of the effluent, but would also widen the C/N ratio to a figure above 15 and render it unsuitable for treatment on a percolating filter. In such cases the addition of increased quantities of soda to induce proteolysis would appear to be the most effective way of overcoming the difficulties.

SUMMARY

Experiments are described showing that the difficulties of direct treatment of milk effluents on percolating filters

are due to the accumulation of fat in the top layers of the filter. Preliminary treatment in a septic tank brings about separation of fat, and thus avoids this difficulty in subsequent treatment on a percolating filter. A rapid lactic acid fermentation also occurs, the effect of which depends on the buffer capacity of the salts in solution. With distilled water containing 1% of milk the reaction of the liquid falls below p_H 4.6 (the isoelectric point of casein) and causes precipitation of casein and inhibition of proteolysis. When hard tap water is used the p_H of the liquid may not fall below 5.5; the casein then remains in solution and undergoes digestion by proteolysis. Such a liquid is quite suitable for treatment on a percolating filter at a rate of 100 gallons per cu. yard per day and leaves behind a tank sludge of low nitrogen content.

The use of alkali in the wash waters also favours proteolysis in the storage tank, but the addition of waste waters containing sugar will result in abnormally high acidities and may cause precipitation of casein even in solutions of high buffer capacity. Such tank effluents especially if of high biochemical oxygen demand have wide C/N ratio and would not be particularly suitable for treatment on a percolating filter without addition of available nitrogen and probably also of phosphates.

Experiments are now in progress in the laboratory and on a large scale on an alternative method (suggested by Whitehead and O'Shaughnessy²) of overcoming the difficulty of ponding which occurs when waste washing waters containing fresh milk are treated directly on a percolating filter. This method requires the use of two filters operated in series. When some solid matter has accumulated in the first filter the order of use is reversed, so that the first filter is supplied with treated effluent from the second filter. This causes the solid matter to disintegrate and oxidise and the filter becomes cleared, after which it may be used again as the first filter when solid matter has collected in the other filter. The success of this method depends on whether the rate of clearing of the one filter proceeds at least as quickly as the rate of accumulation of solid matter in the other filter.

The experiments described in this paper were carried out at Rothamsted as part of the programme of the Water Pollution Research Board of the Department of Scientific and Industrial Research and the results are published by permission of the Department.

Rothamsted Experimental Station,
Harpenden

¹ Ann. Rept. Water Pollution Res. Bd., 1935, p. 27.

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THE BIOLOGICAL OXIDATION OF STEARIC ACID IN PERCOLATING FILTERS

By S. H. JENKINS

Fats of animal and vegetable origin are constituents not only of domestic sewage but also of many trade effluents. For example, fats are contained in the waste waters from dairies and milk products factories, from the scouring of wool, and from the canning of meat and other foods. It is known that fats are decomposed into simpler substances when sewage and trade wastes are treated by biological methods of purification. Few precise experiments seem to have been carried out, however, on the biological decomposition and oxidation of fats in the absence of other organic matter, with the object of obtaining information on the nature and extent of the decomposition and oxidation. Sewage normally contains salts or soaps of fatty acids. Frequently a large proportion of the fat is removed as a calcium soap with the sludge settled by sedimentation, but some of the soap may be carried forward with the settled liquid to be treated by processes of biological oxidation. The experiments described in this paper aimed at providing information on the decomposition and biological oxidation of sodium stearate in percolating filters.

EXPERIMENTAL METHODS

Three filters, *A*, *B*, and *C*, were employed. Each filter, which was 28 in. in depth and 3 in. in diameter, was constructed from four equal lengths of glass tube and was filled with small pieces of glass tubing to serve as the filtering medium. They were arranged as described in an earlier paper,¹ so that samples of effluent from each section could readily be collected for examination. The experiments lasted for 204 days. For the first 17 days the liquids were supplied to the filters at a rate equivalent to 50 gallons per day per cu. yard of filtering medium, for the next 147 days at 100 gals. per day per cu. yard, and for the remaining period of the experiment at rates varying from 20 to 50 gals. per day per cu. yard.

The solution supplied to filter *A* was made up in distilled water to contain sodium stearate equivalent to 25 parts of stearic acid per 100,000 parts, dipotassium hydrogen phosphate equivalent to 1.5 parts of P_2O_5 , ammonium bicarbonate equivalent to 3 parts of N, and small amounts of sodium chloride, calcium chloride, magnesium sulphate, and ferric chloride.

Filter *B* was fed with Harpenden sewage, which had been submitted to preliminary treatment and then supplied with a solution of sodium stearate equivalent to 25 parts of stearic acid per 100,000. The preliminary treatment was necessary as Harpenden sewage contains salts of calcium and has a temporary hardness of about 28 parts per 100,000. Sodium stearate added to the untreated sewage, therefore, is at once precipitated as calcium stearate. According to the method of treatment selected after several trials, 5 litres of crude sewage

were shaken with 1 g. of dipotassium phosphate and the mixture was allowed to settle overnight. The supernatant liquor was then tested by addition of a small quantity of dipotassium phosphate. If a precipitate formed, more phosphate was added until no further precipitation occurred. Sodium stearate added to the sewage softened in this way did not produce a precipitate. In addition to removing calcium, the treatment with potassium phosphate reduced the quantities of suspended and organic matter in the sewage, as shown by the figures in Table I. The comparative results in this table were obtained on examination of crude sewage, supernatant liquid from the same sewage after sedimentation for 16 hours, and supernatant

TABLE I
Composition of crude sewage, before and after settlement, and after treatment with phosphate

	Crude sewage	Crude sewage settled for 16 hrs.	Crude sewage treated with phosphate and settled for 16 hrs.
		Supernatant liquid	
pH value	7.8	7.6	7.4
<i>Parts per 100,000</i>			
Oxygen consumed from $KMnO_4$ in 4 hrs.	12.8	8.0	5.5
Biochemical oxygen demand in 5 days	34.7	29.5	14.4
Suspended solids	8.3	1.6	1.0
Organic carbon	19.1	13.1	7.0
Total nitrogen	7.5	7.0	6.8
N as free and saline ammonia	4.8	4.8	4.8
P, total as P_2O_5	2.25	2.00	about 24
P, inorganic as P_2O_5	0.88	1.13	about 24

liquid after treating the sewage with potassium phosphate and allowing it to settle for 16 hours.

Filter *C* was supplied with crude sewage to which dipotassium phosphate equivalent to 0.75 part of P_2O_5 per 100,000 had been added.

The crude liquids were made up daily and were placed in aspirator bottles from which the filters were supplied. The bottles were thoroughly cleaned once each week. Any solid matter formed in the bottles was allowed to accumulate during the week. The crude liquids and treated effluents from the filters were examined at frequent intervals by the methods of analysis described in an earlier paper.² Effluents from filter *A* were also examined for concentration of stearic acid by the following method. A 150-ml. sample was acidified with hydrochloric acid, evaporated almost to dryness, mixed with cold water, and filtered. The acidified filtrate was evaporated down to a small volume and passed through the same filter-paper, which was then dried. Hot alcohol (98%) was poured on to the filter and the filtrate was evaporated to dryness. The

residue was gently washed with a small volume of cold water; it was then dried at 100° and weighed as stearic acid.

EXPERIMENTAL RESULTS

Biochemical oxygen demand and percentage purification

Results of determinations of oxidisable matter, as measured by the test for biochemical demand in 5 days (B.O.D.) are shown graphically in Figs. 1, 2, and 3. Those for the crude liquid supplied to filter *A* varied considerably although fresh solutions of stearate in the same concentration were added daily to the aspirator. These variations may have been due to the inclusion in some of the samples tested of solid matter which often separated and accumulated until the aspirator was cleaned at the end of each week; or they may have been due to the differences in the number or species of organisms in the incubated samples, although the diluting water used in the test always contained the same quantity of crude sewage—0.5 ml. per litre—as an inoculum. The necessary corrections were made for the diluting water containing sewage. In general, the increases and decreases of the B.O.D. of the treated

for B.O.D. than obtained for the effluents from filter *A*, treating sodium stearate without sewage. Signs of ponding on the surface of filter *B* appeared on the 114th day and remained until the end of the experiment, but the final effluent was clear except on a few occasions.

Filter *C*, with crude sewage only, showed no signs of becoming clogged at any time during the experiment. The quantity of film which collected on the glass medium

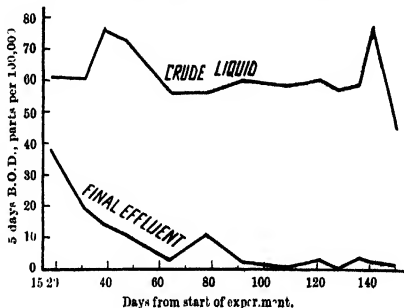


Fig. 2

5 days B.O.D. of crude liquid and final effluent, filter *B*

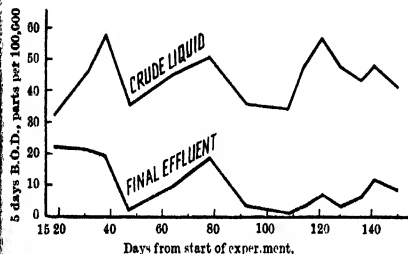


Fig. 1

5 days B.O.D. of crude liquid and final effluent, filter *A*

effluents from filter *A* followed the variations in B.O.D. of the untreated solution of stearate.

Stearate was progressively reduced in amount as the liquid passed through filter *A* but was not entirely removed. For example, the quantities of stearic acid in the crude liquid and in the effluents from sections 1, 2, 3, and 4 on the 47th day were respectively 25, 18.7, 9.5, 3.4, and 2.1 parts per 100,000. The maximum purification by filter *A*, 96%, as measured by B.O.D. in 5 days, occurred on the 108th day. At this stage the average time of contact of the liquid with the filtering medium was about 3 hours. A few tests were made for B.O.D. in 20 days. These tests gave results indicating a lower percentage of purification. For example, the purification on the 108th day, as calculated from the B.O.D. in 20 days, was only 88%.

After filter *A* had been in operation for 114 days it showed signs of becoming clogged and these signs increased until after 164 days white film blocked the perforations in the plate at the bottom of section 3. From the 114th to the 164th day the final effluents were turbid in appearance.

The effluents from filter *B*, which received treated sewage with added stearate, progressively improved during the first 64 days and thereafter gave lower figures

in this filter was much less than in filters *A* and *B*, especially in the upper sections. During the last 106 days the average B.O.D. of the effluent from filter *C* was 2.7 parts per 100,000, or only 0.8 part less than the figure of 3.5 parts for filter *B*, although the crude liquid supplied to filter *C* had a B.O.D. of 21 parts, or about one third of the B.O.D. of 60 parts for filter *B*. After 164 days, however, filter *B* was so badly clogged that it could not treat liquid at a rate of 100 gals. per day per cu. yard, whereas filter *C* showed no signs of ponding.

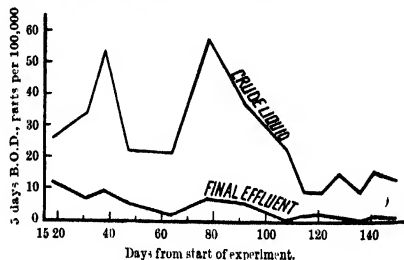


Fig. 3

5 days B.O.D. of crude liquid and final effluent, filter *C*

Average results for the experiments with the three filters are given in Table II.

Progress of purification in sections of the filters

Determinations of B.O.D. in 5 days with effluents from the four sections of the filters were made on occasions with the object of obtaining information on the purification effected as the liquids passed through the filters. The results are given in Table III.

These figures show that in the first section of filter *A* the purification was generally much less than one half of that effected by the whole of the filter, whereas the

first sections of filters *B* and *C* frequently accounted for more than one half of the total purification achieved.

Phosphorus required.—As it has been proved that a deficiency of an available form of phosphorus may restrict the oxidation of substances undergoing treatment in

TABLE II

Average results for biochemical oxygen demand in 5 days in parts per 100,000, and percentage purification

Period	Filter A Sodium stearate solution		Filter B Treated sewage and sodium stearate		Filter C Sewage	
	0-153rd day	47th to 153rd day	0-153rd day	47th to 153rd day	0-153rd day	47th to 153rd day
Untreated liquid B.O.D.	44	44	61	60	25	21
Treated liquid B.O.D.	10	7	8	3.5	4.5	2.7
Percentage puri- fication	78	84	87	94	82	87

percolating filters,³ tests were made at intervals on the effluents from filters *A* and *B*; the results showed the presence of inorganic phosphorus, indicating that the liquids contained sufficient phosphorus for the processes of biological oxidation. The amount added to the

when 0.48 part of nitrogen as nitrite was present in the effluent from filter *C*. Later, both nitrite and nitrate were generally formed in the effluents from filter *B* and *C* and very occasionally in the treated liquid from filter *A*. Some of the results of tests for nitrite and nitrate in the final effluents are given in Table IV.

Film on filtering media.—After 164 days, and until the 204th day of experiment, filters *A* and *B* were supplied with the crude liquids at a rate of flow varying from about 20 to 50 gals. per day per cu. yard; higher rates could not be maintained as these two filters were partially choked. Filter *C*, however, which remained in good condition, was usually supplied until the 204th day with crude sewage at a rate of 100 gals. per day per cu. yard.

The three filters were then dismantled, and the film was removed by washing from the filtering media in the different sections and examined. The microscopical examination of the film was made by Miss A. Dixon of Rothamsted Experimental Station, and the results obtained are shown in Table V.

Analysis of the film for total solids, ash, nitrogen, and stearic acid gave the results in Table VI. The figures in this table represent total quantities in grams in the

TABLE III

B.O.D. in 5 days in parts per 100,000, of untreated liquids and effluents from sections of filters

Day of expt.	31		38		47		92	
	B.O.D.	Purification %	B.O.D.	Purification %	B.O.D.	Purification %	B.O.D.	Purification %
Filter A. Sodium stearate—								
Untreated liquid		46		58		34		36
Effluent section 1		38		33		27		20
" " 2		—		—		21		38
" " 3		—		—		5		85
" " 4		21		20		2.5		93
Filter B. Stearate and sewage—								
Untreated liquid		62		76		72		60
Effluent section 1		35		31		42		41
" " 2		—		—		32		52
" " 3		—		—		23		68
" " 4		19		15		11		74
Filter C. Sewage—								
Untreated liquid		34		54		22		37
Effluent section 1		22		18		12		45
" " 2		—		—		7		68
" " 3		—		—		3.5		84
" " 4		7		9		5.5		75

solution of sodium stearate supplied to filter *A* was 1.5 parts P_2O_5 per 100,000 in the form of dipotassium phosphate.

Compounds of nitrogen in the effluents.—The final effluents in the three filters always contained less ammoniacal nitrogen than in the untreated liquids. For example, on the 47th day the concentrations of ammoniacal nitrogen in the crude liquids and treated effluents, respectively, were: filter *A*, 3.33 and 1.75 parts per 100,000; filter *B*, 5.0 and 1.75; filter *C*, 8.0 and 3.1. The differences varied from time to time. The average amounts of ammoniacal nitrogen removed from the liquids treated over the whole period of the experiments were about 1 part per 100,000 for filter *A* and from 3 to 4 parts for filters *B* and *C*.

Oxidised nitrogen as nitrite or nitrate was not found in any of the treated effluents until about the 47th day,

whole of the film removed from the sections to which the figures refer.

Approximately one half of the dry solid matter of the film in sections 1 and 2 of filter *A*, and about one seventh in sections 1 and 2 of filter *B*, consisted of stearic acid. The film in these sections was almost insoluble in hot water but was readily soluble in hot dilute alkali, from which it could be precipitated on acidification with

TABLE IV

Nitrite and nitrate in treated effluents. Expressed as parts of nitrogen per 100,000

Day of expt.	Filter A		Filter B		Filter C	
	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
64	0	0	0.15	0.71	1.14	3.12
78	0	0	0	3.20	0.36	3.04
92	0.10	0	0.12	0.70	1.00	1.00
108	0.08	0	0.14	1.09	0.24	1.47
121	0	0	0.07	0	0.80	0
136	0	0.32	0.32	2.44	0.40	4.14
141	0	0	0.44	1.56	0.80	9.20

³ Jenkins, *Biochem. J.*, 1935, 29, 116.

hydrochloric acid. It thus appears that the stearic acid was present as such, and not as sodium stearate.

SUMMARY AND CONCLUSIONS

(1) Three percolating filters, *A*, *B*, and *C*, were supplied for several months at a rate of 100 gallons per day per sq. yard of filtering medium with the following liquids:

Filter A.—A solution of sodium stearate containing the equivalent of 25 parts of stearic acid per 100,000

(5) The film on the media in the three filters showed characteristic differences. In the upper half of filter *A* the medium was covered with thick white solid of which approximately one half was stearic acid. The film in the upper half of filter *B* was similar to that in filter *A*, but only about one seventh consisted of stearic acid. In filter *C* the film was a thin brown slime with a flora typical of sewage filters.

(6) It may be concluded that sodium stearate can be

TABLE V

Appearance and results of microscopical examination of film

Section	Filter A	Filter B	Filter C
1	Thick film, white and curdy, with large pink patches: fungal hyphae, clumps of bacteria, wild yeasts (<i>Torula</i> , sp.), <i>Colpoda</i> , <i>Heteromita</i> .	Thick pale brown film with green patches: slimy. Bacteria, yeasts, and algae.	Uniformly brown film, thinly spread over media. <i>Rotifers</i> , <i>Chilodon</i> , nematodes, small <i>Amoebae</i> , flagellates, typical sewage growths.
2	Thick white film as in section 1. More pink growths than in section 1.	Brown film, less than in section 1.	As section 1, but less in amount.
3	Less film than in section 2. Mostly pale brown.	Very little film: brown.	As section 2, but less in amount.
4	Less than in section 3. Same appearance.	As section 3.	As section 3, but less in amount.

besides available nitrogen, phosphorus, potassium, and other essential inorganic salts.

Filter B.—The same amount of stearate as *A* added to sewage from which calcium salts had been removed.

Filter C.—Sewage containing added nitrogen, phosphorus, and potassium salts.

(2) The filters took about 6 weeks to mature and after

oxidised in percolating filters, if available nitrogen, phosphorus, and potassium are present. The oxidation of the stearate occurs more readily in the presence of sewage. With the concentration of stearate used in these experiments the filters may become choked, but this concentration is much greater than likely to be found in any sewage.

TABLE VI

Total solids, nitrogen, and stearic acid in sections: grams

Section	Filter A			Filter B			Filter C		
	Total	Lost on ignition	Ash	Total	Lost on ignition	Ash	Total	Lost on ignition	Ash
1	7.31	0.90	0.41	8.57	6.57	2.00	1.22	0.61	0.61
2	6.16	5.64	0.52	2.28	1.69	0.59	1.29	0.64	0.65
3	2.11	1.80	0.31	2.06	1.64	0.42	0.94	0.44	0.50
4	0.82	0.81	0.01	1.32	1.18	0.14	0.60	0.22	0.38
Total	16.40	15.15	1.25	14.23	08	3.15	4.05	1.91	2.14
Total nitrogen									
1		0.211			0.503			0.046	
2		0.164			0.156			0.050	
3		0.136			0.128			0.039	
4		0.041			0.086			0.022	
Total		0.552			0.873			0.157	
Stearic acid									
1		3.91			0.35			—	
2		2.50			1.20			—	
1 + 2		6.41			1.55			—	

that gave the following average results over a period of 15 weeks:

	Filter A	Filter B	Filter C
Crude liquid, B.O.D. parts per 100,000	44	60	21
Final effluent, B.O.D. parts per 100,000	7	3.5	2.7

The effluent from filter *B* was thus almost as good as that from filter *C*.

(3) Filters *A* and *B* showed signs of becoming clogged after 16 or 17 weeks whereas filter *C* remained in a satisfactory condition.

(4) Nitrite and nitrate rarely appeared in the effluents from filter *A*, but after a few weeks the effluents from filters *B* and *C* regularly contained nitrite and nitrate.

Thanks are due to Mr. E. H. Richards under whose supervision this work was carried out, to Miss A. Dixon for carrying out microscopical examinations, and to Mr. T. Spacey, Manager of the Harpenden Sewage Works, for the regular supply of samples of sewage.

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LXXIV. THE OXIDATION OF AMINO-ACIDS BY HYPOCHLORITE.

I. GLYCINE.

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(Received January 1st, 1936.)

FROM the work of Langheld [1909] with hypochlorite and of Dakin [1916] with chloramine-T it is known that active chlorine may produce from an amino-acid either the aldehyde or cyanide with one less carbon atom together with carbon dioxide and (in the first instance) ammonia. Chlorination of the amino-group has been supposed to constitute the first stage of the reaction. Wright [1926] considered the nature of the reaction to depend on the relative proportions of active chlorine and amino-acid present; with excess of chlorine it was regarded as oxidation and with excess of amino-acid as chlorination.

In the work to be presented, the optimum conditions for the complete oxidation of glycine by hypochlorite have been determined and the course of the reaction studied quantitatively. The investigation arose out of an attempt to use a "chlorine demand" figure for the evaluation of sewage and certain trade wastes.

EXPERIMENTAL.

The reaction between glycine and chlorine was followed iodimetrically. The source of chlorine was the commercial hypochlorite solution of Messrs Laporte Ltd. (Luton), a typical analysis of which showed:

		%
Available chlorine	>15.0
Sodium chloride	13.4
Sodium hydroxide	0.77
Sodium carbonate	0.74

In all experiments, controls consisting of the hypochlorite solutions without glycine were run concurrently. All experiments were carried out at room temperature unless otherwise stated.

(a) *Amount of chlorine used.*

Six series of bottles, each series containing 14 mg. of available chlorine and different amounts of glycine ranging from 1 to 30 mg. in 100 ml. of water, were allowed to stand $\frac{1}{2}$, 1, 2, 3, 4 and 5 hours and the residual Cl titrated. The results for the 2, 3, 4 and 5 hour series were practically identical, indicating that the reaction under these conditions is complete within 2 hours. In Fig. 1 the percentage of available Cl remaining is plotted against mg. of glycine present for the $\frac{1}{2}$, 1 and 2 hour series. The curves are generally similar to those given by Wright [1926], who interpreted the downward portion of the curve as oxidation and the upward portion as mainly chlorination. As an alternative view it seemed possible that glycine in the upward part of the curve was reacting only very slowly.

Several series of oxidations were then run at various levels of glycine. The time allowed was, in every case, 2 hours. The mg. Cl added were plotted against mg. Cl used and a curve obtained for each level of glycine as given in Fig. 2. For 2 mg. of glycine it is seen that when 10 mg. of Cl are added, the curve

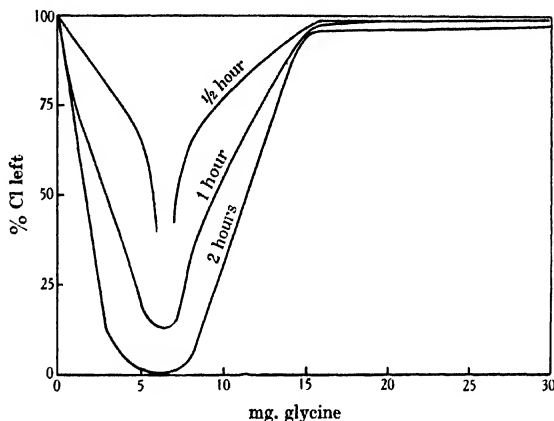


Fig. 1. Percentage of Cl used by glycine in various amounts. 14 mg. chlorine; glycine varied.

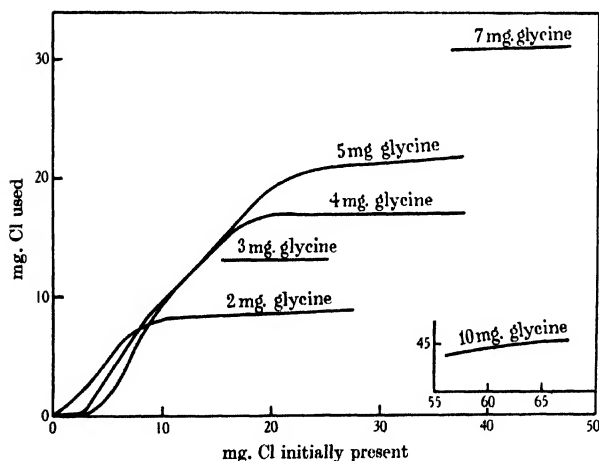


Fig. 2. Quantitative uptake of Cl by glycine.

levels off, indicating that 2 mg. of glycine react quantitatively with 8.55 mg. of Cl. In other words, the presence initially of at least 5 times as much Cl as glycine is necessary before the glycine will use a constant amount of chlorine. Examination of the other curves confirms this, and from them it was ascertained that 1 mg. of glycine uses 4.26 mg. of Cl.

If, for all the values obtained thus far in both experiments (exclusive of the $\frac{1}{2}$ and 1 hour series in Fig. 1 which cannot be considered to have come to com-

pletion), the ratio of Cl initially added to glycine be plotted against the percentage of Cl used, a general curve for the reaction is obtained and shown as the continuous line in Fig. 3.

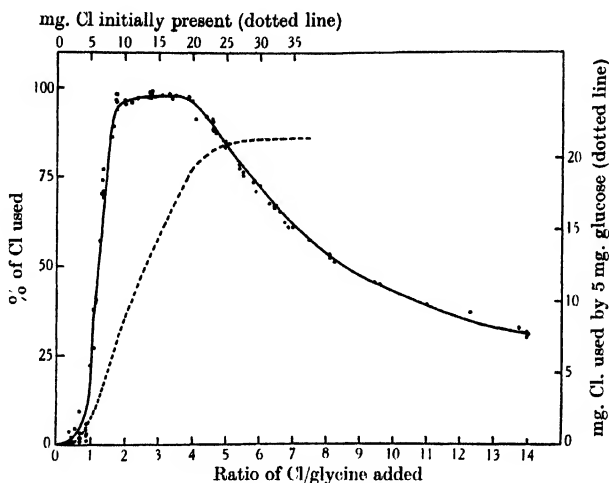


Fig. 3. Effect of Cl concentration.

Table I. *Determination of chlorides.*

A. Variation of Cl with 5 mg. of glycine.

Initial available Cl mg.	Ratio of initial Cl to glycine	Chloride-Cl			One half available Cl used mg.
		At start mg.	After 2 hours mg.	Increase mg.	
14.2 Contr.	—	9.25	9.25	—	—
14.2 Contr.	—	9.25	9.25	—	—
14.2	2.84	9.25	15.9	6.6	6.65
19.9	3.98	12.95	21.9	8.95	9.4
22.7	4.54	14.8	24.7	9.9	10.1
25.5	5.1	16.65	26.9	10.25	10.3
28.4	5.68	18.5	28.9	10.4	10.55
34.1	6.82	22.2	32.8	10.6	10.6
39.7	7.94	25.9	36.6	10.7	10.75

B. Variation of glycine with 14 mg. Cl.

Glycine mg.	Ratio of initial Cl to glycine	Chloride-Cl		One half available Cl used mg.
		After 2 hours mg.	Increase mg.	
Contr.	—	9.0	—	—
Contr.	—	9.0	—	—
1	14.0	11.2	2.2	2.15
3	4.66	15.0	6.0	6.15
4	3.5	15.4	6.4	6.75
5	2.8	15.6	6.6	6.75
7	2.0	15.3	6.3	6.65
8	1.75	14.2	5.2	6.15
9	1.55	13.6	4.6	5.5

C=Control containing no glycine.

(b) Determination of chlorides.

The hypochlorite solution employed contained alkali in the form of sodium hydroxide and sodium carbonate, together with a considerable amount of chloride. When the available Cl is determined by titration in the usual way, not only the Cl actually present in the NaOCl is titrated, but also an equivalent amount of Cl liberated from the chloride, the available Cl being twice the amount of Cl actually present as hypochlorite. If the reaction of Cl with glycine is considered ultimately to be one of oxidation, then for every two hypochlorite Cl atoms or for every four atoms of available Cl two mols. of chloride are formed. In other words, the increase of chloride should be equal to one-half the amount of available Cl used. To test this point, two series of experiments were made in duplicate—one keeping the glycine constant and varying the Cl, the other keeping the Cl constant and varying the glycine. After 2 hours one bottle was titrated for available Cl and the other for chloride. In all cases in which, by reason of the relative proportions of Cl and glycine present, the oxidation was complete (figures in bold type), the increase in chloride agreed well with one half the available Cl used (Table I). When oxidation was incomplete, chloride production lagged behind Cl utilisation.

(c) Factors affecting the rate of reaction.

Physical. Three bottles containing 4 mg. of glycine per 100 ml. and approximately 20 mg. per 100 ml. of available Cl and three controls of hypochlorite alone were set up. One sample and one control were left at 15–17°, one set was placed in a dark cupboard at the same temperature and the other set in an incubator at 30°. 100 ml. were withdrawn from each bottle at various time intervals and titrated for available Cl. All three controls showed no loss of available Cl (Table II).

Table II. *Effect of heat and light on chlorine utilisation.*

Time hours	Incubator, 30° darkness mg. Cl used	Room temp. daylight mg. Cl used	Room temp. darkness mg. Cl used
0.25	0.6	—	0.7
0.5	2.4	1.9	2.2
0.75	5.2	—	4.4
1.0	11.6	6.65	7.15
1.25	16.6	—	13.6
1.5	16.95	15.6	14.8
1.75	17.2	—	15.7
2.0	17.2	16.5	16.4
4.0	—	17.0	17.0

Table III.* *Effect of 500 candle-power light on Cl utilisation.*

Time hours	500 c.p. light mg. Cl used	No light mg. Cl used
0.5	8.55	7.65
0.75	12.75	11.8
1.0	14.5	13.9
1.25	15.8	15.8
1.5	16.5	16.4
1.75	16.9	16.9
2.25	17.2	17.1

* The hypochlorite used in this experiment was from a fresh source, so that the figures are not directly comparable with those of Table II because of alkalinity conditions, which will be discussed later.

The effect of a 500 c.p. light at a distance of 1 ft. on Cl utilisation was tried. The bottle subjected to the light and its control were placed in a glass water-bath in order to eliminate the effect of heat. Another set for comparison was placed in a water-bath of the same temperature but not exposed to the light (Table III).

Chemical. Reaction with hypochlorous acid. The effect of employing free hypochlorous acid instead of sodium hypochlorite was tested. The acid was prepared by passing Cl_2 into a suspension of mercuric oxide and then distilling under reduced pressure. 2 mg. of glycine as the Na salt per 100 ml. water were used. It was found necessary to let the bottles stand longer than the usual 2 hours for completion (Table IV).

Table IV. *Amount of Cl as HOCl used by 2 mg. glycine.*

Cl added mg.	Chlorine used					
	2 hours mg.	5 hours mg.	1 day mg.	2 days mg.	3 days mg.	4 days mg.
3	2.1	2.3	2.35	2.35	2.5	2.45
6	3.6	4.75	5.45	5.6	5.7	5.6
9	4.0	5.0	6.65	7.4	7.6	7.6
15	3.9	5.0	7.05	8.25	9.1	9.05
21	3.65	4.9	7.14	8.4	9.05	9.15

The reaction proceeded very slowly so that 3 days were required. Slightly more Cl (9.05 mg.) was used up by 2 mg. of glycine than was found with sodium hypochlorite (8.55 mg.). The HOCl solution is, of course, acid and completely masks the effect of the small amount of alkali (0.0266 m.mol. per 100 ml.) used to convert glycine into its sodium salt.

Reaction with chlorine water. A similar experiment was carried out with chlorine water. The controls tended to lose Cl at rather varying rates, so at each time interval three control determinations were made and the loss of available chlorine computed from an average of the three (Table V).

Table V. *Amount of Cl used up from Cl water by 2 mg. glycine.*

Initial Cl mg.	Chlorine used				
	2 hours mg.	5 hours mg.	1 day mg.	2 days mg.	3 days mg.
5.5	2.3	3.9	—	—	—
10.95	3.05	4.4	—	—	—
16.4	2.9	4.4	7.1	8.2	8.6
21.9	3.2	4.6	7.3	8.6	8.5
27.4	3.1	5.2	8.1	8.8	8.6

The reaction with chlorine water proceeded slightly faster than with HOCl, the amount of Cl used (8.6 mg. per 2 mg. glycine) agreeing closely with that from sodium hypochlorite.

Effect of acid and alkali on the rate of reaction. The hypochlorite solution employed contained 0.88 % sodium hydroxide and 0.76 % carbonate, exactly 10 ml. of the strong solution being diluted to 500 ml. as the source of chlorine for the following experiments. Large bottles were set up containing 4 mg. of glycine and 10 ml. of the dilute NaOCl solution (containing 20 mg. available Cl) per 100 ml. The acidity of the glycine (4 mg. = 0.0532 m.mol. of acid) and the

alkalinity of the hypochlorite (10 ml. dilute solution = 0.0727 m.mol. of alkali) were taken into consideration and various amounts of H_2SO_4 or NaOH were added so that a range from 25 m.mol. of alkali to 1 m.mol. of acid per 100 ml. of reaction mixture was covered. A large number of controls containing similar additions of acid and alkali were run simultaneously, since in stronger concentrations of acid and alkali the available Cl decreases slowly on long standing. 100 ml. were withdrawn and titrated at various time intervals, the results being given in Figs. 4 and 5. In those cases in which oxidation was complete (Fig. 4) 17 mg. of available Cl were

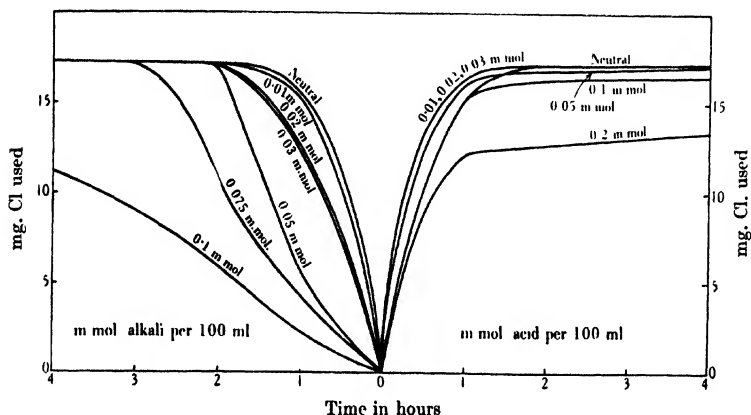


Fig. 4. Effect of acidity or alkalinity on rate of reaction.
4 mg. glycine present per 100 ml.

utilised by 4 mg. of glycine. As the alkali concentration increased, the rate of Cl utilisation decreased. On the acid side the 0.01–0.05 m.mol. reaction mixtures all used Cl at about the same rate, which was slightly faster than in the neutral¹ experiment. After the first hour the 0.1 m.mol. acid sample proceeded at a somewhat slower rate than the neutral one, and the 0.2 m.mol. sample was still slower, not having come to completion in 8 hours. A similar result was obtained with the 0.075 and 0.1 m.mol. alkaline reaction mixtures.

The curves in Fig. 5 represent concentrations of acid and alkali > 0.1 m.mol. per 100 ml. On the acid side, an increase in acid concentration again retarded the rate of reaction. The alkaline side is more complicated for, in contrast with the results in Fig. 4, the greater the amount of alkali the more Cl is used. It seems that small additions of alkali retard the reaction up to a certain point, after which further additions slowly increase the rate of reaction. A few more 2-hour determinations of the amount of available Cl used up were made around what seemed to be the critical point of 0.25 m.mol. of alkali per 100 ml. These, together with those already obtained, are given in Table VI.

This confirmed the fact that the point of reversal due to the effect of alkali was at 0.25 m.mol. of alkali. The slow reactions shown in Fig. 5 were followed for 20 days, but loss of Cl in the controls made interpretation difficult. In general it can be said that the rate of reaction was very slow after a few days in these comparatively strongly alkaline and acid mixtures.

¹ 10 ml. dilute NaOCl contain 0.0727 m.mol. alkali. 4 mg. glycine contains 0.0532 m.mol. acid. 0.0195 m.mol. acid was accordingly added per 100 ml. to neutralise the excess of alkali.

Table VI. *Additions of alkali to a mixture of chlorine and glycine.*

(Cl concentration = 23 mg. per 100 ml. Glycine concentration = 4 mg. per 100 ml.)

m.mol. alkali present per 100 ml.	mg. Cl used 2 hours
—	17.05
0.05	16.85
0.075	10.7
0.1	6.0
0.14	5.7
0.19	5.6
0.22	5.5
0.25	5.15
0.27	5.2
0.30	5.5
0.4	5.6
0.5	6.1
0.7	6.8
1.0	7.6

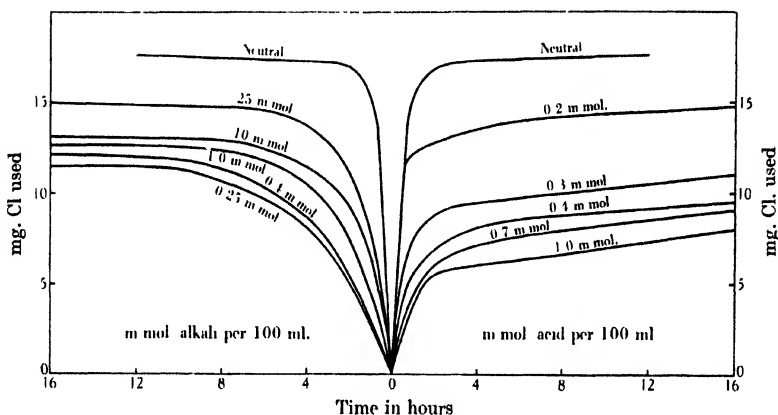


Fig. 5. Effect of acidity or alkalinity on rate of reaction. 4 mg. glycine present per 100 ml.

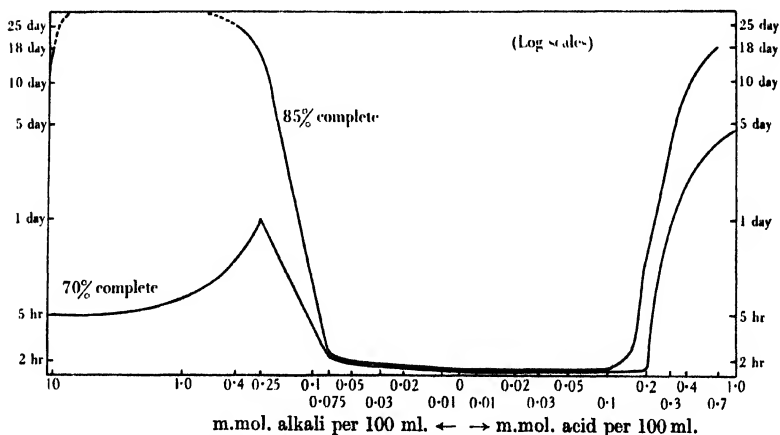


Fig. 6. General variation in rate of reaction.

The general variation in the rate due to the acid or alkali content is, perhaps, best shown in Fig. 6, where the time and the acid or alkali content are plotted on a logarithmic scale. The curves represent 70 and 85 % completion of the reaction, figures which have been arbitrarily chosen. Within the limits of 0.1 m.mol. acid and 0.1 m.mol. alkali per 100 ml. the reaction is rapid, outside these limits, slow, with a reversal on the alkaline side. A curve of a somewhat similar nature was given by Clibbens and Ridge [1927] for the hypochlorite oxidation of cotton.

As a result of these experiments which have shown that very small additions of acid or alkali may greatly affect the rate at which Cl is used by glycine, some former results may be better interpreted. The 2-hour curve in Fig. 1 is very similar to that given by Wright [1926] for 5 hours. Wright stated that he used an alkaline solution of glycine which no doubt retarded the reaction. Similarly the oxidation of glycine by chlorine water and by HOCl was slow, owing to the acidity of these solutions.

Hydrogen ion concentration.

The changes in p_H during the course of the reaction were followed by means of a glass electrode. Three samples, each containing 4 mg. of glycine and 10 ml. of dilute sodium hypochlorite solution (23.0 mg. Cl) per 100 ml. were set up. One received alkali to make the final concentration 0.168 m.mol. alkali per 100 ml., one received no addition of acid or alkali but had present 0.02 m.mol. of alkali per 100 ml. due to the alkali present in the hypochlorite solution, and the third received acid to make the final concentration 0.1 m.mol. acid per 100 ml. The changes in p_H and the Cl used were determined at various time

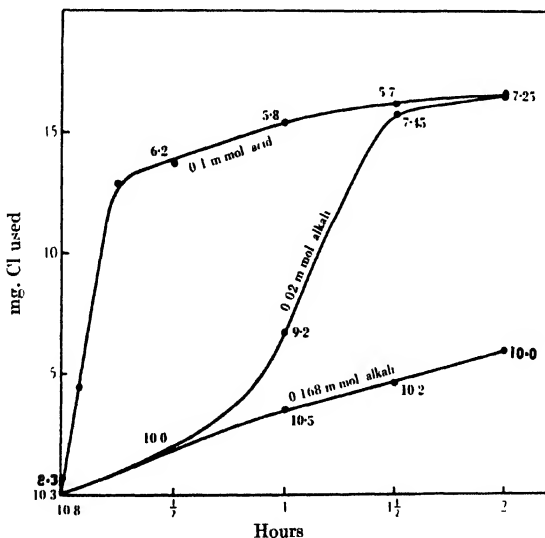


Fig. 7. Effect of p_H on Cl utilisation. Figures in bold type are p_H readings by glass electrode.

intervals. The data presented in Fig. 7 bring out the change in the rate of Cl utilisation due to differences in p_H . The process of oxidation of glycine produces a fall in p_H , the Cl uptake being most rapid between p_H 7.0 and 9.0. Reactions

which were proceeding slowly owing to a high initial p_H were greatly accelerated by suitable adjustment to neutrality (as calculated). Examples are given in Fig. 8, the dotted lines indicating the rate of the reaction if unadjusted.

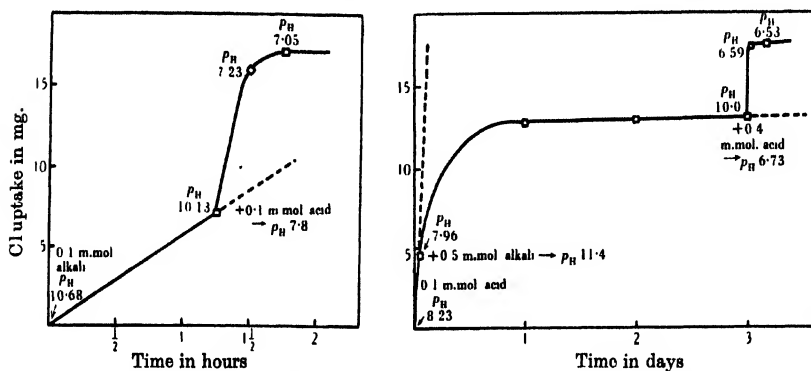


Fig. 8. Effect of adjustment of p_H on rate of oxidation (4 mg. glycine).

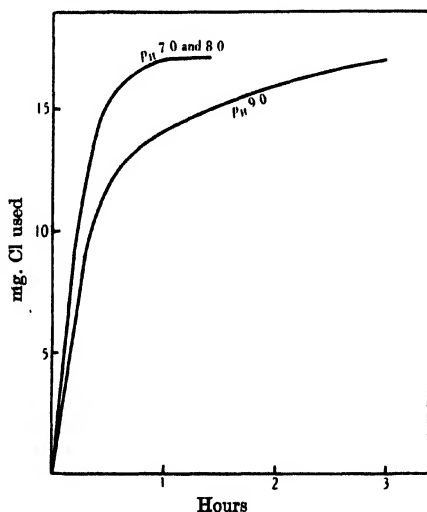


Fig. 9. Utilisation of Cl in buffered solutions.

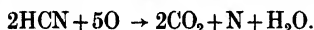
All observations show that, while the reaction may take place partially and slowly when the p_H is high, for complete oxidation approximate neutrality is required; retardation is also evident on the acid side. In order to determine the effect of stabilisation at a given p_H within the optimum range, solutions were buffered with Clark's [1928] phosphate and borate buffers to approximately $p_H 7.0$, 8.0 and 9.0 , and titrated every 10 min. The solutions buffered at $p_H 7.0$ and 8.0 were very similar and are represented by the upper curve in Fig. 9. In the solution buffered at 9.0 , while the initial rate of utilisation during the first half hour somewhat approximated to those in the other solutions, the rate subsequently decreased considerably so that 3 hours were required for complete oxidation of the glycine as compared with 1 hour for the other two.

The route of oxidation.

It has been experimentally determined above that 4.26 mg. of available Cl are utilised in completely oxidising 1 mg. of glycine (8.98 g.-atoms of available Cl per g.-mol. of glycine). Simple chlorination alone could account for but 4 atoms of available Cl. Dakin [1916] stated that the mono- and di-chloroamino-acids decompose spontaneously to give either the aldehyde and ammonia, or the nitrile respectively, CO_2 being liberated in both cases. In the case of glycine, HCHO or HCN would be formed by such decomposition, and using chloramine-T Dakin reported that he obtained traces of HCN from glycine. The uptake of 5 atoms of Cl by glycine remains unaccounted for, and it is evident that there must be a further utilisation of Cl by these initial fission products. Determinations of the amount of Cl used by ammonia, HCHO and HCN were therefore made.

(1) *Formaldehyde*. 2 mg. of formaldehyde were allowed to react with various amounts of sodium hypochlorite solution for 1 hour, 2 hours and 1 day, but in no case was there any loss of available Cl. This excludes the possibility of the formation of formaldehyde as an intermediate product.

(2) *Cyanide*. Solutions containing 1 and 2 mg. of KCN were allowed to react with various amounts of Cl as hypochlorite. The reaction was incomplete in 2 hours, but complete within 24 hours. The titration figures for nine samples varied from 2.8 to 3.15 mg. of Cl used per mg. of KCN present, with an average of 3.0 equiv. to 5.5 atoms of Cl per g.-mol. of cyanide. This is slightly in excess¹ of the theoretical figure of 5 atoms obtained from the following equation:



The reaction, however, is believed to be one initially of hydrolysis to formic acid and ammonia with the subsequent oxidation of these products.



(3) *Formic acid*. Preliminary experiments showed that H.COOH did utilise Cl at a slow rate. 5 mg. H.COOH and 11.2 mg. of available Cl (as sodium hypochlorite) per 100 ml. were allowed to react at room temperature. The amount of Cl used is given in Table VII.

Table VII. *Utilisation of chlorine by 5 mg. of formic acid.*

Time (days)	mg. Cl used
1	3.0
2	7.2
3	7.55
4	7.7
6	7.7

The uptake of 7.7 mg. Cl per 5 mg. of formic acid is equivalent to 2 atoms of Cl per mol. of acid. Evidently the formic acid is oxidised to carbon dioxide and water.

(4) *Ammonia*. 1 mg. of NH_3 was allowed to react with a large excess of Cl (30 mg.) for 2, 3 and 4 hours and 1 and 2 days. Oxidation is rapid and complete in 2 hours. The amount of Cl used is given in Table VIII.

The amount of Cl, 6.75 mg., used by 1 mg. of NH_3 is equivalent to 3.2 atoms of Cl for each mol. of ammonia, which agrees fairly well with the theoretical calculation for complete oxidation to nitrogen and water.

¹ The additional utilisation over and above the theoretical value is believed to be due to the formation of a small amount of nitrite, positive tests for which were obtained in certain cases. This point is being further examined.

Table VIII. *Utilisation of chlorine by 1 mg. of ammonia.*

Time	mg. Cl used
2 hours	6.85
3 "	6.75
3 "	6.75
4 "	6.75
1 day	6.6
2 days	6.65
2 "	6.85
Average	6.75

Thus of the possible intermediate products tested, cyanide, formic acid and ammonia have been shown to react with hypochlorite. The cyanide, most probably through the intermediate formation of formic acid and ammonia, accounts for the utilisation of 2+3 atoms of Cl. This, together with the 4 atoms of Cl required for the initial reaction with glycine, is in agreement with the uptake (by 1 g.-mol. of glycine) of 9 atoms of Cl as experimentally determined.

Carbon dioxide production.

During the reaction, there would be formed therefore, 2 mol. of CO_2 for each mol. of glycine oxidised, if the above theory of oxidation is true. Solutions containing 10 mg. of glycine, 25 ml. of phosphate buffer p_{H} 7.0 and 50 mg. of Cl, added as chlorine water, per 100 ml. of reaction mixture, were kept until the reaction was complete. Chlorine water was used instead of hypochlorite because the latter contained carbonate. After the reaction was complete the CO_2 was aerated off, absorbed in alkali and determined by back-titration. Three determinations gave 11.3, 11.6 and 11.5 mg. of CO_2 . 10 mg. of glycine would give a theoretical figure of 11.7 mg. of CO_2 .

The nature of the initial reaction.

There seems to be no clear evidence as to the nature of the initial reaction between an amino-acid and hypochlorite in excess. Two possibilities exist; either the amino-acid may be chlorinated to give, in the case of glycine, dichloroaminoacetic acid, or the acid may be directly oxidised to form the cyanide, with the liberation of CO_2 and water. Ammonia when treated with hypochlorite does yield chloroamine if the ammonia be present in excess [Chapin, 1929; 1931], an observation which indicates that even under such alkaline conditions hypochlorite may act as a chlorinating agent. Langheld [1909] and Wright [1926] stated that chlorination of the amino-group was the first stage in the action of hypochlorite on an amino-acid or protein in any proportion, and claimed that under certain conditions the dichloro-derivative of a monoamino-acid could be formed by simple mixture of the appropriate quantities of hypochlorite and acid. Since such chloroamino-derivatives are capable of liberating iodine from KI, no apparent loss of chlorine should be observed.

Repetition of Wright's experiment on this point, together with a similar experiment adjusted to neutrality, failed however to confirm his view (Table IX). A slow utilisation of chlorine was observed in the unaltered sample¹ and a more rapid one in the neutralised sample. Controls made at the same time showed a loss of only 0.2 mg. Cl in 3 days and 0.4 mg. in 10 days. These results may be more reasonably explained on the basis of a slow oxidation rather than as the breakdown of an unstable dichloro-compound, which would be expected to be more stable in the neutralised experiment than in the untreated experiment.

¹ Unaltered sample contained 0.17 m.mol. acid.

Table IX. *Utilisation of 14.3 mg. Cl by 15 mg. glycine.*

Time	mg. of available Cl used	
	Unaltered	Neutralised
2 hours	1.0	1.8
5 "	1.2	3.5
17 "	1.8	5.35
1 day	2.1	6.1
3 days	3.7	8.3
6 "	6.15	10.45
10 "	9.0	11.8

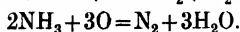
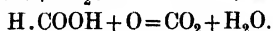
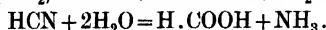
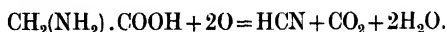
Some other evidence may be cited for the view that ordinary hypochlorite solution does not act as a chlorinating reagent. Cross *et al.* [1908] noted that gelatin treated with chlorine formed a chloro-derivative which may be washed, and subsequently titrated iodimetrically. However, gelatin immersed in hypochlorite retains very little titratable chlorine. Three amounts of 0.2 g. gelatin were allowed to stand with 60 mg. Cl as hypochlorite in 45 ml. water. To one experiment were added 5 ml. *N* acetic acid and to another 5 ml. *N* NaOH, the third receiving only 5 ml. water. After $\frac{1}{2}$ hour the liquid was drained off through fine cloth and the gelatin extensively washed till the washings gave no test for chlorine. This took several hours. The gelatin was then suspended in dilute acidified KI and the iodine liberated titrated in the usual way. The acid sample had retained 16.5 mg. Cl, the alkaline sample 1.4 mg. and the sample with hypochlorite alone 2.5 mg. Calculated on the nitrogen content of the gelatin (17 %) these results correspond to the chlorination of one NH group out of every five in the acid sample, one out of every 66 in the alkaline sample and one out of 37 in the unaltered sample. Chlorination is thus shown to be relatively small, except under acid conditions in which free chlorine would be present.

Indirect evidence against chlorination by hypochlorite is provided by the behaviour of lignified plant tissues after exposure to this reagent. No colour is given on subsequent treatment with cold sodium sulphite solution [Norman and Jenkins, 1933] whereas if acidified hypochlorite or gaseous chlorine be used a characteristic rich purple colour is obtained.

In the light of this rather conflicting evidence the nature of the initial reaction between an amino-acid and chlorine as hypochlorite in excess must remain open. The theory of direct oxidation to cyanide seems at least as well founded as that of the formation of chloroamino-acids.

CONCLUSION.

Whatever be the first step of the reaction, oxidation or chlorination, the reaction between glycine and an excess of hypochlorite probably results in the intermediate formation of hydrocyanic acid, which is then hydrolysed to formic acid and ammonia. These products may then be supposed to be completely oxidised with the liberation of carbon dioxide and gaseous nitrogen. The whole reaction may be given in the equations below, the possible validity of each of which has been experimentally verified.



SUMMARY.

1. Glycine is rapidly oxidised by hypochlorite. At least five times as much chlorine as glycine must be present for completion of the reaction. Under such conditions 1 mg. of glycine uses 4.26 mg. of chlorine, equivalent to 9 atoms of chlorine or $4\frac{1}{2}$ atoms of oxygen per mol. of glycine.

2. When there is a sufficient excess of chlorine to complete the reaction, the increase of chloride-chlorine is equal to one half of the amount of available chlorine used.

3. The rate of oxidation is most rapid and is complete in 2 hours between the acid and alkali concentrations of 0.05 m.mol. per 100 ml. (on either side). In general, further additions of acid or alkali greatly retard the rate of reaction. With alkali however a point of maximum retardation is reached at a concentration of 0.25 m.mol. per 100 ml. In the presence of alkali concentrations greater than this, the rate is very slowly increased, though the reaction is still not complete after a period of 25 days.

4. The change in p_H during the reaction has been followed by means of a glass electrode. The mixture becomes more acid as oxidation proceeds, and the reaction is most rapid in the region of p_H 7-9. Solutions buffered at p_H 7 and 8 are more rapidly oxidised than one buffered at p_H 9.

5. The oxidation of possible intermediate products in the reaction, $H.CHO$, HCN , $H.CO_2H$ and NH_3 , was tested. All except $H.CHO$ are completely oxidised by hypochlorite. From this it is established that the oxidation of glycine results first in formation of HCN , CO_2 and water. HCN is then hydrolysed to give formic acid and ammonia, both of which are oxidised to CO_2 , water and gaseous N . Quantitative recovery of carbon dioxide was obtained. In this way, the uptake of $4\frac{1}{2}$ atoms of oxygen per mol. of glycine is accounted for.

The author is indebted to Sir John Russell for providing facilities for this work, and to Dr M. Starr Nichols of the State Laboratory of Hygiene, Madison, Wisconsin, at whose suggestion it was undertaken.

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AN ECOLOGICAL STUDY OF A THRIPS (*APTINOTHRIPS RUFUS*) AND ITS NEMATODE PARASITE (*ANGUILLULINA APTINI*)

By AVERIL M. LYSAGHT

(*With 7 Figures in the Text*)

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1. INTRODUCTION

(a) *Outline of problem*

It is almost a century now since scientists began to set out long-term experiments to elucidate the causes of differences in vegetation such as occur, for example, in the clearly defined zones on mountain slopes. The classical fields

at Rothamsted are valuable from this point of view. In 1856 the meadowland which comprises Park Grass was divided into strips for experimental work on the effect of fertilizers on grassland. Since then the plots have undergone differential manurial treatments and have been annually cropped for hay. Differences in the vegetation and sometimes in the insect population of these plots have become apparent. The present investigation was undertaken in order to determine, if possible, what factors influenced the distribution of the parasites of an insect which occurs on all the plots.

A wingless Thysanopteron, *Aptinothrips rufus* Gmelin, is abundant throughout the year on the grasslands at Rothamsted. It is parasitized by a nematode *Anquillulina aptini* (Sharga). Sharga (unpublished observation), working on samples from two of the plots, found that the parasite was apparently absent from one although common on the other. The two plots have had similar manurial treatment except that one has been dressed with lime while the other has not.

In 1933-5 observations concerning the distribution of both the host and parasite on eleven of the plots were made at intervals to see whether the difference in abundance of the parasite was significant or fortuitous.

I wish to thank Sir John Russell for his kindness in allowing me to work at Rothamsted Experimental Station. The investigation was carried out in the Entomology Department under the stimulating direction of Dr C. B. Williams. To him and to the other members of the staff who have discussed the questions involved and who have been generous with helpful criticism I am most grateful. Thanks are also due to Mr Samuel and Mr Moore who kindly undertook the culture of a fungus parasite of *Aptinothrips rufus*, to Mr P. Simon, without whose assistance the experiments involving water cultures and the growing of a number of grasses could not have been carried out, and to Messrs Sutton and Son who supplied varieties of grass seed for experimental purposes.

(b) *Description of the grass plots*

(1) *Manurial treatments*

The material from which the insects were collected for examination was obtained by sampling a number of plots from the field called Park Grass. This field was divided into plots for experimental purposes in 1856 (Lawes & Gilbert, 1880-1900). The plots used in the present investigation were chosen so as to give as great a range as possible of manurial treatment and of resultant variation in the herbage. They vary in size from one-half to one-twelfth of an acre. Their relative positions and treatments are shown in Fig. 1. Each plot is divided into two halves, similarly treated save that the south-west half of each has been limed regularly since 1883, and the other half left unlimed. No lime was applied at all before that year.

(2) Chemical data

Recent data on chemical analyses of the soils have been kindly supplied by the Chemistry Department and are given in Table 1: the plots differ considerably and present a wide range of pH values.

	Limed	Unlimed	
13	X	X	14 tons dung alternating with 6 cwt fish meal Dressings every two years
	↔ (49 acres)		
12	No lime		Unmanured (both halves)
11 ²			As 11 ¹ with 400 lb. silicate of soda
11 ¹		X	As 7 with 618 lb. sul. am. (129 lb. N)
10			As 9 without potash
9	X	X	As 7 with 412 lb. sul. amm. (86 lb. N)
8			As 7 without potash
7	X	X	3½ cwt. super., 500 lb. sul. pot., 100 lb. sul. mag., 100 lb. sul. soda
6			Sul. amm. (86 lb. N) for first 13 years, then as 7
5 ²	Super + potash after sul amm (86 lb N) for 42 yrs. No lime	5 ¹	Unmanured after sul. amm. (as 5 ²)
4 ²			3½ cwt. super., 412 lb. sul. amm.
4 ¹			3½ cwt. super.
3	X	X	Unmanured
2			Unmanured after dung for first 8 years
1			206 lb. sul. amm. (86 lb. N)
14	X	X	As 7, with 550 lb. nit. soda (86 lb. N)
15			Minerals as 7, following nit. soda first 18 years
16			As 7, with 275 lb. nit. soda (43 lb. N)
17			275 lb. nit. soda (43 lb. N)

"X" indicates that a plot has been sampled for
Aptenothrips rufus

Fig. 1.

Table 1

Plot...	3 L	3 U	7 L	7 U	13 L	13 U	9 L	9 U	14 L	14 U	11 ¹ U
A	7.3	5.6	6.7	5.1	6.6	4.7	5.0	4.0	7.2	6.0	3.8 (1923)
B	91	4	100	0	99	30	21	-1	108	105	—
C	21	25	32	39	45	32	28	31	38	10	—
D	—	1.51	1.20	1.31	1.09	1.18	1.00	1.37	1.07	1.07	—
E	—	0.18	0.43	0.11	0.55	0.61	0.68	0.77	0.55	0.60	—

U = unlimed plot.

L = limed plot

A = pH values for 1933, 2 years after liming

B = percentage nitrification (1933).

C = average mineralizable nitrogen (1933).

D = percentage nitrogen in herbage (hay (1931), with 80 % dry matter).

E = cwt. nitrogen per acre in hay (1931)

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Data for 11¹ U are not given here as it has not been subjected to chemical or botanical analysis since 1924 and it was not included in the present investigation till 1934. Its herbage is almost identical with that of 9 U but its pH value is slightly lower (3.8 in 1923).

An attempt was made to correlate these and other values with the percentage infection of *Aptinotrips rufus* both by the nematode and by a parasitic fungus (Lysaght, 1936 *b*). None of the correlations was significant and certain factors were thus eliminated.

(3) *Botanical data*

Unfortunately an exact botanical analysis of the plots is not available. The most recent was published by Brenchley in 1924 since when considerable changes have taken place. At that time the main constituents of the herbage on 9 U were *Agrostis vulgaris*, *Anthoxanthum odoratum*, *Arrhenatherum avenaceum*, *Festuca ovina*, *Holcus lanatus*, *Dactylis glomerata* and *Rumex*

Table 2

Limed	Unlimed
	Plot 3
<i>Avena pubescens</i>	<i>Avena pubescens</i>
<i>Briza media</i>	<i>Briza media</i>
<i>Dactylis glomerata</i>	<i>Anthoxanthum odoratum</i>
<i>Anthoxanthum odoratum</i>	<i>Festuca ovina</i>
<i>Festuca ovina</i>	<i>Conopodium denudatum</i>
<i>Poa pratensis</i>	
<i>Trifolium pratense</i> (v. ab.)	
<i>Ranunculus acris</i>	
	Plot 7
<i>Avena pubescens</i>	<i>Avena pubescens</i>
<i>Anthoxanthum odoratum</i>	<i>Anthoxanthum odoratum</i>
<i>Dactylis glomerata</i>	<i>Dactylis glomerata</i>
<i>Briza media</i>	<i>Briza media</i>
<i>Taraxacum officinale</i> (v. ab.)	<i>Trifolium pratense</i>
	<i>Conopodium denudatum</i>
	<i>Alopecurus pratensis</i>
	<i>Poa pratensis</i>
	Plot 9
<i>Holcus lanatus</i>	<i>Holcus lanatus</i> (v. ab.)
<i>Anthoxanthum odoratum</i>	<i>Anthoxanthum odoratum</i> (sc.)
<i>Alopecurus pratensis</i>	
<i>Bromus mollis</i>	
<i>Poa pratensis</i>	
	Plot 11 ¹ U
	<i>Holcus lanatus</i>
	Plot 13
<i>Alopecurus pratensis</i>	<i>Alopecurus pratensis</i> (v. ab.)
<i>Dactylis glomerata</i>	<i>Dactylis glomerata</i> (sc.)
<i>Arrhenatherum avenaceum</i>	<i>Arrhenatherum avenaceum</i> (sc.)
<i>Holcus lanatus</i>	<i>Conopodium denudatum</i> (sc.)
	<i>Rumex acetosa</i> (sc.)
	Plot 14
<i>Arrhenatherum avenaceum</i>	<i>Arrhenatherum avenaceum</i> (v. ab.)
<i>Anthoxanthum odoratum</i>	<i>Alopecurus pratensis</i>
<i>Dactylis glomerata</i>	<i>Bromus mollis</i>
<i>Poa trivialis</i>	<i>Avena pubescens</i>
<i>Poa pratensis</i>	

acetosa. In 1928-9 the winter was exceptionally hard and the only grass able to withstand both the extreme cold and the high acid content of the soil was *Holcus lanatus*. This plot is now almost covered with *H. lanatus* but there is a little *Anthoxanthum odoratum* as well. Plot 11¹ U is rather similar but even in 1924 *Holcus lanatus* was dominant. Occasionally *Anthoxanthum avenaceum* has been plentiful and a few other species could always be found. Now however *Holcus lanatus* makes up the entire herbage and the surface is a thick peaty mat.

The growth on 9 U and 11¹ U is coarse and rank. The latter yields a heavier crop of hay than any other in this series. It produces on an average 58 cwt. per acre from the first cut while the rest yield from 11 to 52 cwt. The herbage on all the other plots consists of a number of species, and though sometimes one may be particularly abundant, in no case does it dominate the rest of the association. A list of the commoner species which were in flower in May and June 1933 is given in Table 2. It is by no means complete but is compiled from eye observations carried out by the Botany Department.

(c) *Technique*

(1) *Sampling*

Samples were taken at intervals of about one week throughout the spring and summer of 1933. In 1934 they were taken weekly from February till November, and then at intervals of from 2 to 3 weeks until the end of February 1935. As the grass was cut on each plot it was put into paper bags and then brought back to the laboratory for examination. Each sample was left in a large glass jar covered with muslin until I was ready to work through it (not more than 3 or 4 days).

Aptinotrips rufus is about 1.25 mm. in length, wingless, and yellowish brown as its name denotes. It can be distinguished quite easily from the other thrips found with it since it is more slender than the pale larval forms of the commoner winged species and has a peculiar gait. Specimens were at first collected with a camel-hair brush but later an aspirator was used. Various methods were used for killing the insects, but anaesthetizing with chloroform proved to be the most satisfactory. If, however, too much vapour were present the blood coagulated rapidly and the parasites degenerated almost immediately. A simple device was suggested to me and proved effective. A wide-necked bottle of chloroform was fitted with a rubber cork in which two holes were bored. The glass tubing from the bottom of the aspirator containing the thrips was fitted through one hole, while through the other was passed a piece of bent glass tubing to which a rubber bulb was attached. Pressure on the bulb forced chloroform vapour up through the aspirator and in this way a very small amount could be used.

Dissections were made in Ringer's solution. Centrifuging of washings from the grass of a heavily infested plot were carried out in the summer of 1934 in

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the hope of finding the free-living stages of *Anguillulina aptini*, but the only nematodes collected were free-living and saprophytic forms.

(2) *Culturing the nematode*

The fact that Glaser (1932) and Lapage (1933 *a*) successfully cultivated two widely different types of nematodes suggested that it might be possible to obtain cultures of *A. aptini*. Attempts were made to do this using Glaser's technique. The nematodes were placed on agar plates inoculated with a heavy suspension of baker's yeast, but none lived for more than 2 days although alive and active at the end of 24 hours.

2. BIOLOGY OF THE NEMATODE

(a) *Life cycle*

The adult female nematode although degenerate in many respects has an extremely efficient reproductive system. One parasitized thrips was found to contain a single female nematode, 111 eggs and 18 newly hatched larvae. Other instances are given in Table 3. I obtained no evidence concerning the length of the egg-laying period in individual females.

Table 3. *Typical infestation of individual thrips. "Male larvae" includes only those which are sufficiently advanced to be recognizable on account of their caudal alae*

Females	Eggs	Larvae	(Male larvae)
2	56	133	29
2	33	122	5
2	84	113	4
3	62	122	1
1	43	125	9
1	111	18	0
1	30	113	1
2	145	9	0

The maximum production of eggs is in the spring during the months of April and May (Fig. 2 and Table 4). From the relationship of the curves showing the average numbers of larvae and eggs in infected thrips in 1933 and in 1934-5 it would appear that the incubation period in the spring is between 3 and 4 weeks. After the middle of May the number of eggs drops appreciably but it rises again at the beginning and at the end of July. The curve then slopes fairly smoothly down and reaches its minimum in December. It fluctuates about a low level throughout the winter months and shows a steady rise in March.

It is possible that the drop in May is due to the fact that the overwintering generation of females has died out and that their progeny have not yet reached maturity. After this period development is probably accelerated by the rise in temperature. That this is so is confirmed by the fact that the numbers of eggs and larvae per infected thrips undergo a progressive decrease from the end of

June onwards in both 1933 and 1934, while, on the other hand, in neither year does the curve showing percentage of infected thrips (Fig. 3) reach its maximum till August and September.

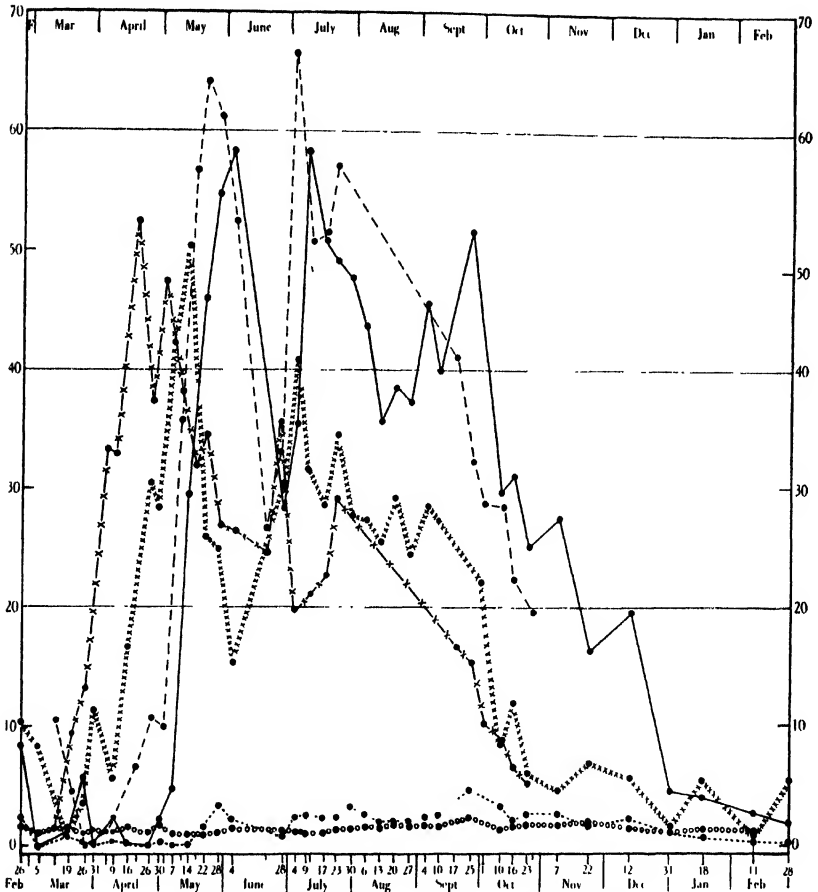


Fig. 2. Average number of nematodes per infected thrips.

	1933	1934-5
Eggs	—x—x—x—	—x—x—x—x—
Larvae	— — — —	— — — —
Females	—o—o—o—o—	—o—o—o—o—
Male larvae	—x—x—x—x—	—x—x—x—x—

It is not known whether moulting occurs before the larvae leave the thrips. I think that the young males and females escape from the host early in the morning when the dew is heavy and swim down to the shelter of the grass sheaths and that here moulting occurs and fertilization takes place. On the other hand fertilization may occur in the host before the larvae leave the anus.

Table 4. *Average numbers of nematodes per thrips in weekly samples from all plots*

Date 1933	Number of thrips infected	Average number of nematodes per thrips			
		Males	Females	Eggs	Larvae
13 Mar.	2	2.5	0.0	1.5	10.5
21	11	0.4	0.5	9.6	4.7
27	4	0.0	0.8	13.3	0.0
6 April	8	0.0	0.9	33.5	1.3
10	15	0.0	0.9	32.9	2.5
20	11	0.0	1.2	52.5	6.7
27	9	0.0	1.6	37.2	10.9
2 May	8	0.0	1.0	47.4	10.0
11	17	1.1	1.1	38.1	35.8
18	19	3.2	1.0	32.1	56.8
22	39	4.9	1.3	34.6	64.3
29	27	3.8	1.0	27.0	61.1
5 June	23	2.9	1.5	26.6	52.3
20	23	1.3	1.2	24.7	26.9
26	31	1.3	1.2	35.7	33.2
3 July	55	2.5	1.1	19.9	66.6
11	51	2.5	1.3	21.2	50.8
18	54	2.7	1.4	22.9	51.6
23	60	2.6	1.4	29.3	57.1
18 Sept.	96	2.6	1.9	16.9	41.2
26	47	3.9	1.8	15.5	32.3
2 Oct.	21	3.1	1.9	10.3	28.9
11	18	4.3	3.3	8.9	28.6
16	12	2.3	1.8	6.5	21.3
26	22	2.2	2.0	4.8	19.6
1934					
26 Feb.	5	2.4	1.6	10.2	8.4
5 Mar.	7	0.0	1.0	8.6	0.0
19	6	6.7	1.5	0.8	1.2
26	14	0.3	1.1	3.7	5.8
31	13	0.0	1.5	11.5	0.4
9 Apr.	6	0.3	1.2	5.8	2.3
16	9	0.0	1.6	16.7	0.2
26	19	0.0	1.2	30.5	0.0
30	16	0.4	1.8	28.6	2.1
7 May	20	0.0	1.2	42.4	4.8
14	13	0.0	1.0	50.5	29.5
22	16	1.8	1.1	26.2	45.9
28	20	3.4	1.1	25.1	54.8
4 June	10	2.2	1.2	15.4	58.2
28	20	0.9	1.1	30.4	28.4
4 July	11	2.5	1.5	41.0	37.6
9	23	2.7	1.0	31.8	58.4
17	42	2.6	1.2	28.6	50.7
23	40	2.6	1.3	34.6	49.3
30	41	3.4	1.4	27.8	47.6
6 Aug.	54	2.7	1.7	27.1	43.7
13	65	2.0	1.5	25.5	35.7
20	60	2.2	1.8	29.3	38.6
27	73	2.2	1.8	24.3	37.3
4 Sept.	36	2.5	1.8	28.8	45.3
10	42	2.5	1.7	27.5	40.2
25	44	4.8	2.4	22.1	51.6
10 Oct.	14	3.3	1.4	8.6	29.9
16	28	2.2	1.7	12.1	31.1
23	29	2.8	1.8	6.1	25.2
7 Nov.	22	2.7	1.8	4.7	27.6
22	19	1.7	1.8	7.1	14.4
12 Dec.	15	2.3	1.5	5.7	17.6
31	16	1.1	1.2	1.7	4.5
1935					
18 Jan.	19	0.6	1.5	5.4	4.0
11 Feb.	17	0.4	1.4	0.8	2.8
28	19	0.2	1.2	5.7	2.0

Note. Numbers in the column headed larvae include those which were distinguishable as males as these were thought to be immature.

This hypothesis is supported by the comparatively small number of male larvae which are found throughout the summer (Fig. 2). Males have, however, been found leaving the anus of the host.

After fertilization the female finds a larval or pupal thrips and enters it by means of the stylet which enables it to penetrate the delicate skin of the host. Bovien (1932) has seen females of *Scatonema wülkeri* Bovien penetrating the larvae of *Scatopse fuscipes* Meig. in this way. The female nematode after entering the host loses its worm-like appearance and develops into a swollen sac-like organism in which the reproductive system is the only obvious structure (Lysaght, 1936 a).

I think that the last generation of females in the autumn infects the larval thrips which will form the overwintering generation. Development of the female nematodes in these hosts is delayed and few eggs or larvae are found throughout the winter months. The number of females per infected thrips was practically constant during the entire period of sampling. The examination of 1650 infected thrips showed that in the great majority one or two females occurred in each host, but occasionally more were present, up to eight in very rare cases.

Fig. 2 shows the average number of parasites per host insect in 1933 and 1934-5. Numbers of females in 1933 are not shown as the curves for both years are almost identical.

(b) Experimental work

Experiments were carried out to ascertain whether the life cycle of the nematode could be completed in the absence of soil. Grass seedlings which had been grown in sterilized soil were carefully washed and placed in water cultures. Cellophane cages were fixed over them. Six tubes containing *Holcus lanatus* and six containing mixed grasses were used. The mixed grasses comprised *Agrostis alba*, *Alopecurus pratensis*, *Bromus mollis*, *Dactylis glomerata*, *Festuca ovina* and *Poa pratensis*. Ten thrips from 13 U were placed in each cage. The percentage infection of thrips was 78 % on this plot on 27 August 1934, and 45 % on 4 September, 3 days before this experiment was begun.

The thrips were placed on the mixed grasses on 8 September and on *Holcus lanatus* on 7 September, and were left till 22 October when they were collected for examination. The results are given in Table 5. The cages were left undisturbed during this period, but the solution in the tubes was changed weekly. The grasses did not grow well and in three sets of *H. lanatus* the seedlings died. From the other three 39 thrips were collected. Of these, 35 were larvae, 2 were pupae and 2 adults. One larva only was infested: an immature female was present in its body cavity.

The mixed grasses were much more healthy but only 18 thrips were present, these comprised 4 pupae, 7 larvae and 7 adults. The adults were small and pale. Of these 18, 5 were infected, 4 adults and 1 pupa. This shows that it is possible

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(under certain conditions) for the nematodes to complete their life cycle without utilizing the soil. All the available evidence points to the grass sheaths being the habitat of the nematodes in the free-living stage.

Table 5

Grass	Thrips				Nematodes			
	Pupae	Larvae	Adults	Infected specimens	Males	Females	Eggs	Larvae
<i>H. lanatus</i> (a)	—	6	2	1 la.	—	1 (imm.)	—	—
(b)	2	19	—	—	—	—	—	—
(c)	—	10	—	—	—	—	—	—
Mixed (a)	—	—	1	1 ad.	—	2	15	—
(b)	1	1	2	1 pu.	—	2	2	103
	—	—	—	1 ad.	5	2	29	22
(c)	1	—	2	1 ad.	—	1	35	1
(d)	—	—	1	1 ad.	1	1	—	—
(e)	2	3	—	—	—	—	—	—
(f)	—	3	1	—	—	—	—	—

(c) *Seasonal variation in abundance of the nematode*

(1) *Effect of temperature*

The graphs for the nine plots seem to exhibit a fairly regular seasonal rhythm. Infection rises gradually in April and May, drops in June, reaches a maximum in August and September and drops at the end of October. In the mild winter of 1934-5 a mid-winter rise occurred on three of the plots, but this did not persist through February. The approximately weekly average of the percentage infection on all the plots for 1934-5 is shown in Table 7 and Fig. 4. The peak was reached on 27 August.

The monthly average of the percentage infection on all the plots in 1933 and 1934-5 is expressed in Table 6 and Fig. 3. The curves for both seasons are almost identical. Samples unfortunately could not be taken in August 1933, and it is probable that infection reached its peak during that month. This would explain the only discrepancy between the curves.

Table 6 and Fig. 3 shows the monthly mean of the minimum temperature on grass and it is obvious that a relationship exists between the rise in temperature and infection.

Correlations were worked out to estimate the significance of this relationship. The average minimum temperature on grass for 1 week before sampling and the percentage infection of that sample gave a correlation coefficient of 0.66, a highly significant figure (0.3809 was required). The average minimum temperature on grass for 1 month before sampling gave an even more significant correlation, 0.7321. The highest correlation coefficient was 0.7528, which was obtained from the correlation of the percentage infection with the average temperature on the screen for one month before sampling. These figures are of undoubted interest but their true significance is difficult to judge in the absence of laboratory experiments under controlled conditions of humidity and temperature. A significant correlation can usually be obtained

Table 6

Average number of thrips per sample on all plots, 1934-5		Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Percentage infection on each plot for 1933		30-8	24-0	47-8	36-8	25-4	54-1	32-3	23-6	58-4	77-7	82-2	46-5	50-6
14 U 14 L 13 U 13 L (11 ¹ U)	14 U	—	1-7	7-2	10-1	4-3	8-6	—	40-0	16-5	—	—	—	—
	14 L	—	5-7	6-3	5-7	7-3	7-7	—	17-0	6-3	—	—	—	—
	13 U	—	1-0	1-2	3-2	3-9	11-9	—	17-0	2-0	—	—	—	—
	13 L	—	0-9	1-2	1-9	0-8	5-0	—	17-5	7-0	—	—	—	—
	(11 ¹ U)	—	—	—	—	—	—	—	—	—	—	—	—	—
9 U 9 L 7 U 7 L 3 U 3 L	9 U	—	0-0	0-6	0-5	0-0	0-5	—	0-0	1-0	—	—	—	—
	9 L	—	0-9	8-6	6-7	4-7	18-1	—	29-4	17-9	—	—	—	—
	7 U	—	1-5	1-3	6-0	7-7	13-8	—	10-3	7-0	—	—	—	—
	7 L	—	4-9	7-9	9-1	2-9	23-4	—	7-9	3-0	—	—	—	—
	3 U	—	3-0	11-6	11-6	21-6	40-0	—	17-7	11-5	—	—	—	—
Percentage infection on each plot for 1934-5	3 L	—	5-8	8-3	11-2	9-9	31-7	—	13-0	3-0	—	—	—	—
	14 U	0-0	8-4	12-0	9-0	7-5	17-7	42-0	55-0	33-7	20-0	14-0	32-0	26-0
	14 L	0-0	7-5	6-0	4-7	5-6	12-6	21-0	34-7	14-7	14-0	6-0	16-0	8-0
	13 U	0-0	5-0	3-0	9-0	10-0	24-0	64-0	38-7	13-3	12-0	4-0	4-0	4-0
	13 L	0-0	7-1	1-0	2-1	4-0	20-8	30-9	23-6	9-3	8-9	4-0	4-0	12-0
9 U 9 L 7 U 7 L 3 U 3 L	11 ¹ U	0-0	0-0	0-0	0-0	2-0	0-7	1-1	0-0	0-0	0-0	0-0	0-0	2-0
	9 U	0-0	0-0	0-0	2-0	0-0	0-7	4-4	1-0	0-0	2-0	0-0	0-0	2-0
	9 L	2-5	4-0	11-0	12-0	10-0	14-7	35-6	31-3	6-3	12-0	20-0	0-0	4-0
	7 U	4-0	4-9	3-7	7-6	9-1	3-2	8-7	5-3	2-7	6-0	0-0	0-0	4-0
	7 L	8-0	1-3	2-4	7-0	0-0	8-0	21-7	12-3	9-3	4-0	0-0	4-0	6-0
Percentage infection on all plots monthly for 1933	3 U	0-0	6-0	12-7	13-0	14-0	12-0	22-2	11-5	4-0	0-0	10-0	4-0	8-0
	3 L	3-3	0-0	9-6	4-0	4-0	11-1	22-8	6-5	4-3	6-0	2-0	8-0	2-0
	—	—	2-2	4-8	6-9	6-1	10-9	—	17-9	7-4	—	—	—	—
	—	1-3	4-0	5-2	6-4	5-9	11-6	26-2	21-9	8-9	7-5	5-6	6-9	7-1
	—	—	30-3	33-9	41-0	44-0	50-9	48-6	46-5	38-8	—	—	—	—
Average monthly temp. in ° F. (min. on grass) for 1933		—	—	—	—	—	—	—	—	—	—	—	—	—
Monthly sums of rainfall in mm. (8 in. gauge) for 1933		—	2-19	0-73	0-92	0-08	1-10	0-46	1-92	1-09	—	—	—	—
Average monthly temp in ° F. (min. on grass) for 1934-5		—	26-8	34-8	38-3	44-8	47-2	45-3	43-9	39-6	34-7	36-7	31-7	32-5
Monthly sums of rainfall in mm. (8 in. gauge) for 1934-5		—	11-48	47-2	21-25	42-25	27-05	44-95	67-25	47-65	44-45	122-4	21-05	67-3

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between temperature and the population of most animals that breed in the summer. Rise of temperature is only one of a number of complex factors that together constitute the change of environment at this season.

(2) *Effect of rainfall*

That rainfall might affect the nematode attack was considered possible but the correlation between infection and total rainfall for a month before sampling was negative and not significant. It would seem that dew provides sufficient moisture for the nematodes to complete the free-living stages of their life cycle.

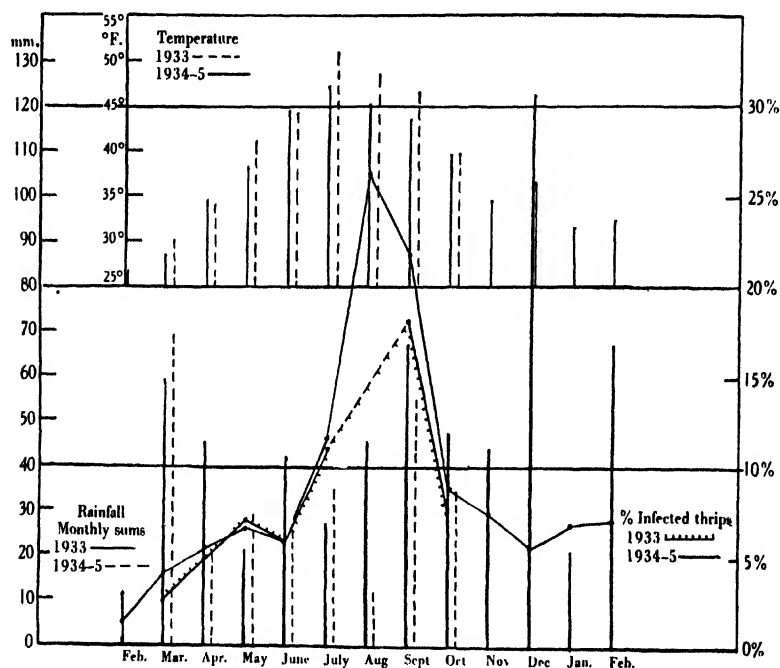


Fig. 3.

Heavy rain would most likely tend to wash them off the grass into the soil. Rainfall in relation to infection is shown in Figs. 3 and 5 and Table 7.

(3) *Total numbers of parasite and host*

A final point of some interest in the question of infection is raised by the population figures for both host and parasite plotted in Fig. 4 and Table 7. The abundance of the different stages of the nematode was estimated by multiplying together the average number of thrips in the weekly samples, the percentage of infected thrips and the average numbers of larvae or eggs found in an infected thrips. Presumably this would give a fairly accurate figure for the nematodes present on the volumes of grass in each sample.¹ The curve

¹ 4-lb. jars, capacity 2400 c.c.

expressing numbers of nematode larvae in a certain volume of grass attains its maximum in mid-July. At the same time the second brood of thrips reaches its peak (see Section 3 *a* and *b*). The average minimum temperature on grass is higher in July than at any other time. The percentage of thrips infected does not reach its maximum till 6 weeks later when the larvae of the second brood of thrips have attained maturity.

Table 7

Date 1934	Average population of thrips per sample 1934-5	Per- centage of infected thrips	Calculated numbers of nematodes per sample		Rainfall before sampling (in mm.)	
			Larvae	Eggs	28 days	14 days
26 Feb.	31	1	2.48	3.16	6.48	2.60
5 Mar.	23	3	0.00	5.93	12.99	8.50
19	28	2	0.56	0.45	55.35	42.65
26	29	5	8.70	5.37	66.50	38.35
31	16	7	0.00	2.88	57.40	13.55
9 Apr.	28	2	1.12	3.25	42.25	3.90
16	37	4	0.00	24.72	22.35	7.80
26	61	8	0.00	48.84	30.40	24.65
30	64	6	7.68	109.82	47.20	39.40
7 May	50	7	15.00	148.40	57.60	44.70
14	44	5	63.80	111.10	55.10	15.70
22	26	6	71.76	40.87	46.05	6.05
28	27	7	103.95	47.44	21.25	5.55
4 June	16	4	37.12	9.86	6.95	0.90
28	35	7	68.60	74.48	35.45	29.00
4 July	35	5	66.50	71.75	42.15	34.50
9	57	10	397.10	181.26	38.50	27.15
17	72	12	440.64	247.10	44.65	14.30
23	54	16	423.36	298.94	52.95	31.00
30	52	16	399.36	231.30	29.75	20.65
6 Aug.	34	23	344.08	211.92	32.45	9.35
13	36	25	324.00	229.50	36.25	15.60
20	31	26	314.34	236.16	21.15	11.80
27	27	31	309.69	203.39	20.95	5.35
4 Sept.	25	19	209.00	136.80	58.05	48.65
10	27	22	237.60	163.35	52.45	47.10
17	23	21	*	*	75.90	27.25
25	19	20	197.60	83.98	62.05	39.70
1 Oct.	30	*	*	*	21.45	19.10
10	74	5	111.00	31.82	—	—
16	52	11	177.32	69.21	—	—
23	77	11	211.75	51.67	—	—
7 Nov.	80	8	179.20	30.08	—	—
22	76	7	74.48	37.77	—	—
12 Dec.	80	5	72.00	22.80	—	—
31	84	6	25.20	8.06	—	—
1935						
18 Jan.	46	7	12.88	17.71	—	—
11 Feb.	68	6	12.24	3.26	—	—
28	33	7	4.62	13.17	—	—

* No dissections.

The various generations of thrips overlap as the adults appear to live for some time. Taking Fig. 2 in conjunction with Fig. 4 it would appear that the high temperature in July greatly accelerates the rate of development of the nematodes and appears to increase their chance of survival in the free-living

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stage. The numbers of infected thrips therefore show a steady increase after the July maximum although the number of eggs and larvae produced by the female nematodes undergoes a comparatively steady decline. The ideas suggested by the data expressed in these graphs are put forward with some diffidence as so many factors are involved.

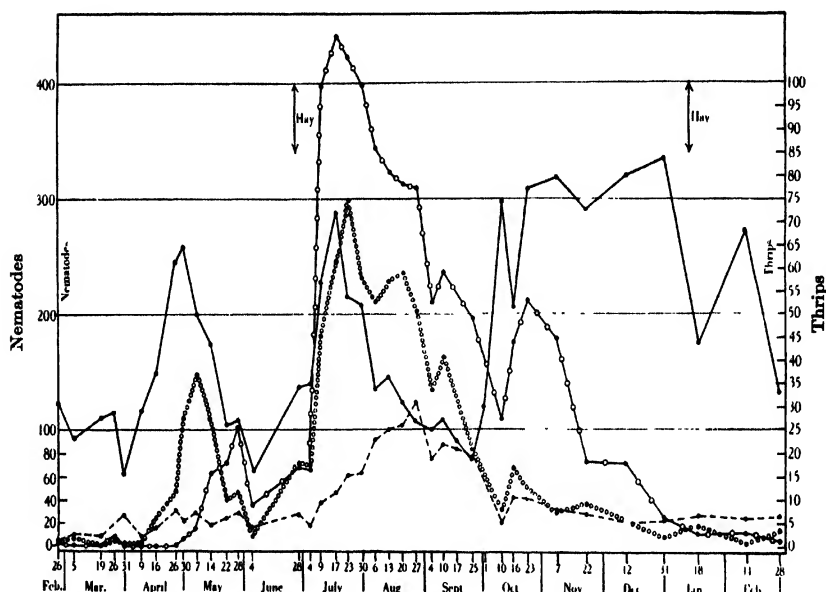


Fig. 4. Abundance of thrips and nematodes on all plots, 1934-5. Thrips (10-min. catches) —●—, infected thrips (%) — — —. Nematode eggs ○○○○○○○○○, nematode larvae ○—○—.

(d) *Morphological effect of parasite on host*

The effect of parasitism on the host is that sterility almost always follows. Amongst all the infected thrips dissected only 8 were found in which the reproductive organs appeared to be normal (Table 8).

Table 8

Date	Nematodes per thrips				Eggs of thrips
	Males	Females	Eggs	Larvae	
21. iii. 33	—	—	22	12	1
21. iii. 33	(1)	2	17	6	2
26. vi. 33	—	—	4	—	1
23. vii. 33	—	1	9	—	Ovaries well developed, no mature eggs
20. vi. 33	—	1	7	—	Mature eggs
17. vii. 34	(8)	—	—	18	1
31. iii. 34	—	1	—	—	1
13. viii. 34	—	—	—	2	1

It would be conceivable that, if the pupal host were parasitized at a late stage in its development, the eggs and ovaries would not at first be affected by the presence of the parasites. Sterility follows as a rule when only one immature parasitic female is present, but in three of the above cases the infection is fairly heavy. These three thrips were perhaps attacked in the late pupal stage. Very little is known about the exact effects of parasitism in insects.

3. VARIATION IN NUMBERS OF HOST INSECT

(a) Evidence

An estimate of the thrips population on all the plots was attempted in 1934. In 1933 I had noticed that they varied in number very considerably but I had no standard method to measure their abundance. The method used in

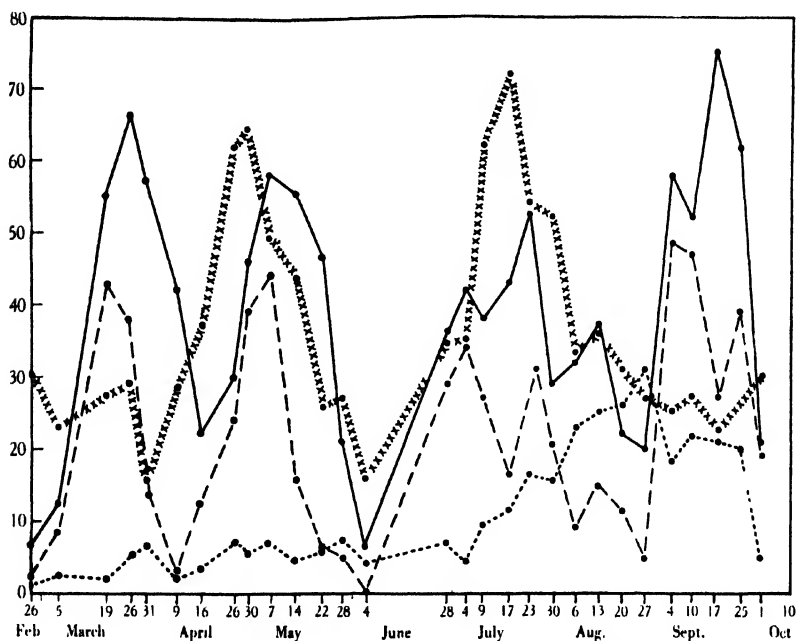


Fig. 5. Average numbers of thrips per sample xxxxxxxx; percentage nematode infection -----; total rainfall in mm. 28 days before sampling ———; total rainfall in mm. 14 days before sampling — · — ·.

1934 was to collect as many thrips as possible in 10 min. from samples of grass of about the same volume (2400 c.c.). This method was used because the amount of herbage on the manured and unmanured plots varied so much that it was impossible to take the grass from a given area. On a piece of ground 5 ft. square on 9 U in the late summer thousands of thrips would have been present and only a few score on the same area on 3 U and 3 L. On the whole

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the thrips were much more abundant on some plots than on others but seasonal increases in the population on all of them were very similar.

Table 9

	Plot 3 U	Plot 3 L	Plot 7 U	Plot 7 L	Plot 9 U	Plot 9 L	Plot 11 ¹ U	Plot 13 U	Plot 13 L	Plot 14 U	Plot 14 L
1934											
26 Feb.	9	14	21	17	33	33	40	60	50	33	26
5 Mar.	6	14	18	12	28	31	9	45	26	24	41
19	8	16	10	14	42	26	31	54	50	32	23
26	4	8	20	15	54	21	46	52	32	40	30
31	4	4	8	5	25	14	45	18	20	15	16
9 Apr.	7	5	11	17	50	26	52	54	33	29	29
16	3	12	14	11	53	47	66	72	35	52	42
26	7	6	18	28	59	102	71	107	108	70	100
30	17	10	39	28	60	102	75	102	81	97	96
7 May	26	24	31	31	86	72	27	80	67	37	68
14	22	21	21	21	42	66	53	63	35	21	64
22	22	35	17	22	57	26	37	27	19	12	14
28	23	15	15	24	30	55	43	35	22	23	15
4 June	13	20	6	18	21	32	16	16	18	9	9
28	18	23	18	37	56	46	34	36	38	21	53
4 July	3	18	9	21	64	63	85	28	42	22	33
9	8	5	14	35	78	59	183	52	82	78	34
17	11	8	19	36	146	82	236	64	36	98	56
23	23	9	17	9	104	58	165	65	27	19	99
30	7	10	8	8	130	33	125	50	30	98	55
6 Aug.	17	6	8	18	58	25	76	42	22	32	68
13	5	7	9	18	86	36	35	32	13	94	66
20	17	9	2	20	58	25	60	22	8	72	52
27	25	7	9	11	56	17	38	37	10	38	53
4 Sept.	11	5	8	5	58	12	58	11	19	46	46
10	6	4	4	15	68	14	66	30	9	51	35
17	14	4	12	10	68	5	56	17	8	39	16
25	18	11	5	13	27	21	41	21	11	24	16
1 Oct.	12	16	21	24	81	32	63	27	9	31	17
10	26	22	21	38	223	128	126	88	62	51	34
16	40	19	18	34	127	45	58	68	47	83	30
23	31	72	16	46	192	105	106	66	45	110	61
7 Nov.	42	58	25	57	163	111	109	77	62	96	78
22	49	41	35	24	119	103	128	85	44	130	72
12 Dec.	45	57	47	105	150	82	120	78	65	62	74
31	73	79	54	81	98	108	42	98	40	112	137
18 Jan. 1935	9	25	24	23	110	52	97	63	56	37	14
11 Feb.	38	24	32	46	148	73	106	68	40	90	84
28	13	12	34	21	44	47	77	16	26	11	34
	730	755	718	1018	3152	2035	2901	2026	1447	2039	1890

Numbers of thrips found in 10-min. counts in laboratory samples.

The figures for individual plots from 26 February 1934 to 28 February 1935 are given in Table 9 and the monthly and weekly averages compiled from these are shown in Table 7 and Figs. 4, 5 and 6. More thrips occurred on 9 U and 11¹ U than on any other plots, 3152 on 9 U and 2901 on 11¹ U. These figures were significantly higher than the rest which varied between 718 on 7 U and 2039 on 14 U.

(b) *Effect of parasitism*

I do not think it probable that the absence of parasites on 9 U and 11¹ U is responsible for the higher population figures on these areas. Knechtel (1923) reports that *Aptinothrips rufus* was present in great numbers on the ears of *Holcus lanatus* in Roumania. It appears to be a favourite food plant and its

tufts provide plenty of shelter. *Aptinothrips rufus* seems to prefer a certain amount of shelter and has been found to be less abundant on the tops of the grasses.

Fig. 4 shows that the number of thrips which begins to decrease in late July reaches its lowest point on 25 September. The peak of infection by the nematodes was reached in August. At first sight therefore it would appear that parasitism might be affecting the population. This is improbable for two reasons. First, 9 U and 11¹ U both show a considerable drop in their population

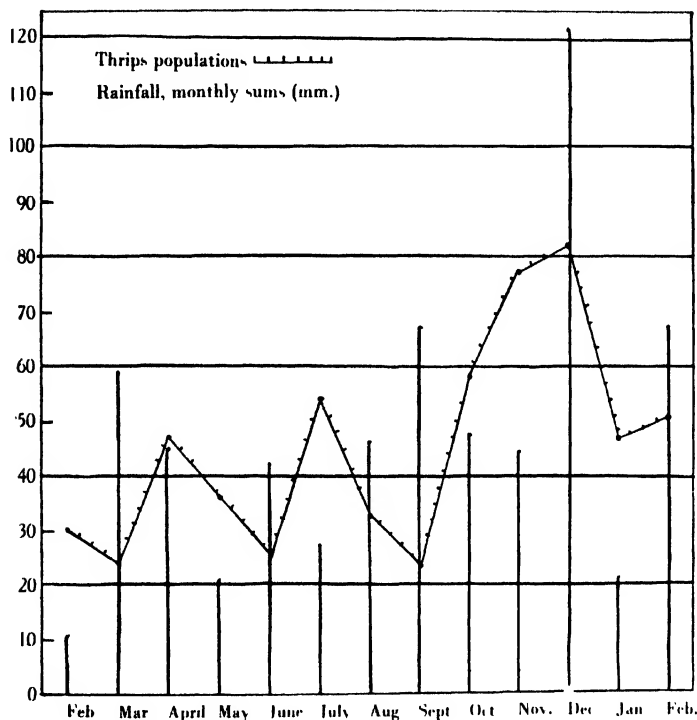


Fig. 6

figures for September. Secondly, the thrips show three peaks, one in April, a second in July and a third in the winter, extending from October to January. These three peaks are separated by deep depressions which are of fairly uniform depth, that in September being slightly shallower than the other two and these can have no connexion with nematode attack. It is therefore highly probable that the consistently higher population on 9 U and 11¹ U is due to the fact that *Aptinothrips rufus* prefers *Holcus lanatus* to the other grasses.

Aptinothrips rufus was bred on various grasses for experimental purposes but little difference was apparent in either the numbers or the vigour of the F_1 generation.

4. DIFFERENCE IN PERCENTAGE PARASITISM ON ELEVEN PLOTS

(a) *Evidence*

The present investigation was undertaken primarily to ascertain whether the apparent absence of the nematode on some plots was real and permanent. In 1933 the evidence proved that there was a significant difference in infesta-

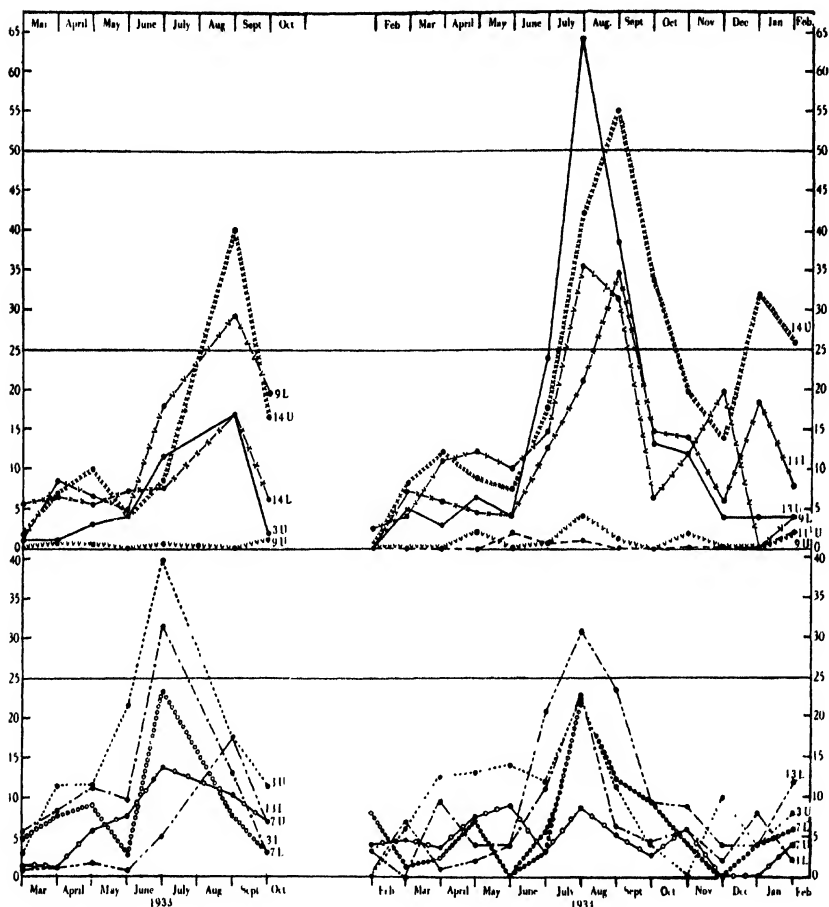


Fig. 7. Monthly percentage infection of thrips by nematodes. Plots 3 U; 3 L ————; 7 U ————; 7 L ————; 9 U ————; 9 L ————; 11 U ————; 13 U ————; 13 L ————; 14 U ————; 14 L ————

tion between 9 U and the other plots. In 1934 11¹ U was also sampled. Its vegetation is similar and its pH slightly lower. The percentage of thrips infested by nematodes is rather less than on 9 U.

Fig. 7 and Table 6 show the monthly average of infection on all the plots from 1933 to 1935. The highest infection on 9 U for any one month was 4 %

in August 1934, while in 1933 it failed to rise above 1 %. On 11¹ U it rose to 1 % in June 1934 and February 1935. In only 4 of 13 months was any infection found on this plot.

On each of the other nine plots infection rose to a marked degree during the summer months. On 13 U a heavy infestation occurred rising to 68 % in August 1934. On 7 U it was much lower, the highest point for 1934 being 8.5 % and for 1933 14 %. However if the curve for this plot be compared with those for 11¹ U and 9 U it will be seen to be consistently higher.

On comparing the infestation of any plot in 1933 with that in 1934 it is seen (Fig. 7, Table 10) that there is much variation and that infestation may be high in one season and low in another. In July 1933 infection on 3 U rose to 40 % but in 1934 only to 22 %. The curve for 3 L was somewhat similar though in 1933 it did not reach as high a maximum as 3 U. 7 L showed almost identical curves for both years but the maximum was reached a month later in 1934. 9 L also shows similar curves for both seasons. In 1933 infection on 13 U failed to rise above 17 %, but in 1934-5 it was higher than on any other and reached a monthly average of 64 % in August. This figure was taken from four weekly samples each over 60 %. The 1933 peak infection was thus quadrupled in 1934. 13 L with a maximum of 17.5 % in 1933 almost doubled this number in 1934, and 14 L shows a similar variation. 14 U on the other hand had a fairly high infection in 1933, a 40 % average in September; in 1934 a maximum of 55 % was reached in September, but, unlike the other plots, infection remained high throughout the winter months and reached an average of 32 % in January and 25.5 % in February, 1935. In February 1934 no infection was found on either 14 U or 14 L.

Table 10

Plot	1933		1934	
	Maximum % infection	Month	Maximum % infection	Month
3 U	40.0	July	22.2	Aug.
3 L	31.7	July	22.7	Aug.
7 U	13.8	July	8.7	Aug.
7 L	23.4	July	21.7	Aug.
9 U	1.0	Oct.	4.0	Aug.
9 L	29.4	Sept.	35.6	Aug.
11 ¹ U	—	—	2.0	June
13 U	17.0	Sept.	64.0	Aug.
13 L	17.5	Sept.	30.8	Aug.
14 U	40.0	Sept.	55.0	Sept.
14 L	17.0	Sept.	34.7	Sept.

From these observations it is obvious that the curve of infection fluctuates considerably throughout the seasons on all plots but that the range is much less on 9 U and 11¹ U.

(b) *Chemical factors*

The pH values (Table 1) for 9 U and 11¹ U are lower than for any other plot, but, on the other hand, the calculated correlation between pH values and infection over all the plots was not significant. In 1933 the correlation coefficient was 0.325 and 0.632 was required for this set of figures; in 1934 it was 0.332 while 0.632 was needed.

There has been a tendency in recent years to regard the pH of a soil as the most significant factor present. It must be remembered that pH is only one of a number of very highly complex factors influencing soil populations. Arrhenius (1921), working with soil samples with a pH value varying from 3-10, found that the earthworm *Perichaeta indica* Horst was able to survive only in soil with a pH of 6 or 7. As a result, perhaps, of his work there seems to have been a general belief that pH was important as a factor in controlling nematodes (Emmert, 1931). Vague references to such a theory occur quite frequently. Smith (1929), working on the relationship between physico-chemical properties of soil and abundance of *Heterodera* cysts, experimented with 78 soil samples and concluded that in the case of sandy soils there was no correlation. In peaty soils however he found a significant negative correlation between pH and cyst counts. Godfrey & Hagan (1933), working on soils with a pH varying from 3.5 to 8.5, found no correlation between pH and infection by *H. radiculicola*, and Chandler's (1925) hookworm larvae were able to survive immersion in N/10 solutions (pH 1) of HCl for 18 hours when the temperature did not rise above 75° F.

I worked out correlations in order to estimate whether any significant relationship existed between the presence of *Anguillulina aptini* and the percentage of nitrogen in the herbage, weight of nitrogen per acre in the hay, percentage nitrification and the average mineralizable nitrogen but none of these was significant.

(c) *Botanical factors*(1) *Experiments*

The growth of grass on the different plots varies according to the manurial treatments. Thus, in the plots under discussion, 11¹ U, 9 L and 9 U, 13 L and 13 U, 14 L and 14 U produce the heaviest crops of hay while the herbage on 7 U and 7 L is not nearly so rank. Both 3 U and 3 L produce very little grass and the thrips are much less abundant on these two as a rule. The lists of grasses given in Table 2 show that they are very mixed on most of the plots. *Arrhenatherum avenaceum* is very abundant on 14 U, *Alopecurus pratensis* on 13 L, but the only plots where one species is dominant are 9 U and 11¹ U. In 11¹ U *Holcus lanatus* has taken complete possession while on 9 U this species is dominant but a little *Anthoxanthum odoratum* is to be found.

It is known (Triffitt, 1934) that the secretions of some grasses influence the development of the eggs of *Heterodera schachtii* and I thought it possible that

Holcus lanatus might either harm the nematode by its secretions or hinder it from finding a host by its long and numerous hairs. To test this, two 6-in. cubes of turf were taken from each of the plots 9 U and 9 L and were placed in earthenware pots in the greenhouse. After fumigation with hydrocyanic gas they were examined and no living thrips could be found. On 1 May 50 thrips were placed on each lot of grass. The pots were treated as follows: 9 U A and 9 L A were both used as controls and 50 thrips from the original plots were placed on each. On 9 U B were placed 50 thrips from 9 L and on 9 L B 50 thrips from 9 U.

On 3 August the grass was cut and 25 thrips were taken from each of the four plots. Only 2 of these were infected by nematodes, 1 in 9 L A and 1 in 9 U B. In this one case, at any rate, it seemed possible for a nematode to complete its life cycle on *Holcus lanatus*. Such a small infestation was unsatisfactory, and at the beginning of September more thrips were taken from the plots and put on the grass. On 22 October 30 were taken from each pot and examined. The results are tabulated in Table 11. On neither pot infested with thrips from 9 U was there any infection. On the control pot 9 L A 23 % of the thrips were infected and on 9 U B (infected from 9 L) 20 %. It might be argued that the original thrips had survived and that no infection of the next generation had taken place but in that event one would expect to find a heavy infection in each insect whereas in only 3 out of the 13 were larval nematodes abundant and males present; the majority contained females and eggs with very few larvae.

These results are strong evidence against the theory that any secretion from *H. lanatus* inhibited the development of the nematode.

Table 11

Experiment	No. thrips ex.	No. thrips inf.		Nematodes			
				Males	Females	Eggs	Larvae
9 U A (control)	30	0		—	—	—	—
9 U B	30	6	(1)	—	2	—	—
			(2)	—	1	9	14
			(3)	—	1	—	—
			(4)	—	4	—	—
			(5)	—	1	6	2
			(6)	—	2	—	5
			(7)	—	4	—	—
9 L A (control)	30	7	(1)	—	4	—	—
			(2)	1	1	13	62
			(3)	1	1	14	26
			(4)	—	4	—	—
			(5)	—	3	2	—
			(6)	1	2	—	26
			(7)	—	4	—	15
9 L B	30	0					

The pH values of the soils were taken at the end of the experiments and are as follows, 9 U A 3.65, 9 U B 3.95, 9 L A 5.15, 9 L B 6.4. These gave additional proof that a low pH was not a directly important factor in preventing the nematodes from completing their development.

(2) *Discussion*

It appears that some conditions were present in the greenhouse experiments which were not found on the plots, and the two most obvious of these are the rankness of *Holcus lanatus* on 9 U as opposed to the more delicate growth under greenhouse conditions, and secondly the high humidity which exists in the cellophane cages in the greenhouse.

H. lanatus in midsummer on 9 U is extremely coarse and the sheaths of its stems are of great length. Short lengths of stems have been kept in the laboratory in Petri dishes and thrips of varying age have been placed on them. Almost invariably the insects sheltered in the curling edges of the blades and seemed unable to penetrate into the sheath for more than a few mm., if at all. In the field it is probable that the larvae and pupae do not penetrate into the sheath at all but shelter at the base of the blade just where it leaves the sheath. The nematode is much smaller than the host and it may make its way down into the sheath for a considerable distance. If that is so it may have difficulty, when it has matured, in finding the host. There are so many more species of grasses on the other plots that even if some, such *Dactylis glomerata*, have long rank sheaths there are still numerous finer and smaller grasses where these conditions would not be present. In *H. lanatus* the hairs on the leaf hold rain in large drops and prevent its running down into the sheaths; the sheaths of more open grasses with less velvety leaves are likely to have a higher humidity.

In the greenhouse experiments growth was never as vigorous as it is under external conditions and the grass was cut on several occasions. Also in the cellophane cages humidity is exceptionally high. Actual measurements have not been carried out; but dew remains on the grass till much later in the day than it does on the grasses just outside the greenhouse. The nematode which was seeking a host in any of the greenhouse cages would have a short distance to travel on account of the small size of the grass, while the excessive humidity would be an added advantage and give it a longer period to seek the host without danger of desiccation.

At present it is not known for how long the female nematode can survive without finding the host. Development may occur within a short time of fertilization, or alternatively it may be delayed till some kind of stimulation is set up by the blood of the host. It would appear that there is a definite critical period at this stage.

It should be noted that the possibility of the absence of infection on 9 U and 11¹ U being due merely to geographical position was raised, but as infected thrips occur round the edges of 9 U this does not appear to be a likely factor. Spreading from one plot to another must occur by means of wind and mechanical agents, but this cannot take place to any extent in view of the definite differences between adjacent plots.

(d) *Parasites other than the nematode*

Unidentified *Trombidium* larvae have been found firmly attached to the hind coxa of a number of specimens of *Aptinothrips rufus* during the late spring. Ten were found in May and one in mid-July. In no instance did they appear to have exerted any malign influence on the host. The ovaries appeared normal and the insects healthy and active.

Spores of an unidentified fungus were found in the body cavity of *A. rufus*. Thirty-four insects were found to be infected: sterility seems to result.

5. DISCUSSION

The observations made on the thrips population over a short period indicate that parasitism by the nematodes does not appear to exert a profound influence on the abundance of the insects.

The effect of the parasite on individual insects suggests that the next step in the investigation should be the culturing of the nematodes in hanging drops. This presents numerous difficulties which, however, should not be insurmountable. If the free-living female nematodes could be obtained thus it should be possible to infect larval and pupal thrips and so gain information about the causes of sterility. This may be due to a toxin or it may be due to the absorption of protein by the nematode. According to Norris (1933, 1934) most adult insects need protein for the normal development of their genital products.

Biological control extends over such a large field that, although *Aptinothrips rufus* is not itself of economic importance, much fundamental information might be gained by continuing the investigation along these lines.

6. SUMMARY

1. *Aptinothrips rufus* Gmelin is abundant on the grass plots of the classical field Park Grass at Rothamsted Experimental Station. Sampling has been carried out for 2 years on a number of the plots and population counts have been made.

2. *A. rufus* is parasitized by *Anguillulina aptini* (Sharga). Some experimental work on the biology of the nematode has been carried out.

3. The nematode is rarely found on two of the plots and this difference in distribution was found to be constant during 1933 and 1934. There is a very rank growth of *Holcus lanatus* on these two plots and the coarseness of this growth appears to exert an unfavourable effect on the nematodes. Infected insects have been bred on a less sturdy growth of *H. lanatus* under greenhouse conditions.

4. An account is given of other factors which might affect the distribution of the nematode.

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METHODS OF INVESTIGATING THE BIONOMICS OF THE COMMON CRANE-FLY, *TIPULA* *PALUDOSA* MEIGEN, TOGETHER WITH SOME RESULTS

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(With Plate XXVII)

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I. INTRODUCTION

It was desired to incorporate the study of the common injurious crane-fly, *Tipula paludosa* Meigen, into the "Studies of Fluctuations in Insect Populations" carried out by the writer in recent years (1). A consideration of the means whereby this could be done revealed the fact that the present state of knowledge of the bionomics of the crane-flies was inadequate, especially as regards the identification of the immature stages. It was therefore decided to start a study of the bionomics of the more common and injurious species. The present paper deals with attempts to find suitable methods and the results obtained.

II. THE METHODS USED TO OBTAIN THE PRELIMINARY STOCK MATERIAL

As a preliminary to starting pure stocks from adult crane-flies, larvae were obtained by watering grassland, known to be infested, with an emulsion of orthodichlorobenzene.¹ This is a well-known method and very simple to use. The appropriate solution is applied with a watering-

¹ The St Ives leatherjacket exterminator, an emulsion of orthodichlorobenzene and Jeyes fluid, was used at normal strength, applying it at the rate of 1 gallon per sq. yard.

can with rose to the turf and in a few minutes the larvae (leatherjackets) come to the surface. If they are not collected within about 10 min., they burrow again back into the soil.

The site selected was the strip of lawn (80 yards long by 1 yard wide) alongside the ditch and hedge running roughly north and south in the home apiary at the Rothamsted Experimental Station, Harpenden. This area was divided into ten randomized blocks of 8 sq. yards, 1 sq. yard per block being treated each week for a period of 8 weeks, in order to obtain an adequate idea of the distribution of the larvae in the site selected.

The period of the year chosen for the treatment was based on the fact that *Tipula paludosa* is known to be on the wing from July until September. The sampling was started on 13 May and ended on 29 June. The numbers of larvae obtained each week are shown in Table I. Full details of the numbers obtained per sample are omitted. However, Mr W. G. Cochran of the Statistical Department, to whom the writer is indebted for the lay-out of the plots, has examined the data and found that the sampling was sufficiently accurate to detect a difference from year to year of ± 30 per cent in the total population of the 80 plots. Towards the south and more shady end of the grass strip a definite increase in numbers was found.

Table I
*Number of leatherjackets obtained 13 May to 29 June 1936
by orthodichlorobenzene method*

Date of sampling	Number of leatherjackets obtained
13. v. 36	127
19. v. 36	91
26. v. 36	45
2. vi. 36	39
9. vi. 36	21
16. vi. 36	16
22. vi. 36	10
29. vi. 36	22

It will be seen that the numbers of larvae obtained steadily decreased from the first application of the emulsion. From this it is suggested, after studying the dates of emergence of the adult crane-flies (16 August to 14 September) and allowing for a 3 weeks pupal period,¹ that the larvae of *T. paludosa* are not susceptible to this treatment for a period of at

¹ Oldham states (*Proc. R. phys. Soc. Edinb.* xxi, 1929, pp. 217-52) that the pupal period of *T. paludosa* averaged 10 days (max. 13, min. 8), while Balachowsky (*Les Insectes nuisibles aux plantes cultivées*, I, 1935, p. 865) states 3 weeks. Probably the period is nearer 2 weeks than 3.

least 6 weeks prior to pupation, though this needs to be checked by further experiments. In other words, it is suggested that the curve of numbers of larvae obtained by the treatment in this particular instance is a picture of the numbers of larvae becoming non-susceptible to the treatment and also a curve parallel to that of pupation.

III. METHOD USED IN REARING ADULT CRANE-FLIES FROM FULL-GROWN LARVAE

A week previous to the first sampling, ten small flower pots were sown heavily with wheat and in each successive week a further ten pots. It was thus possible to place the larvae from each sample (square yard) immediately in a pot of germinating wheat. Originally it was intended to preserve half the larvae from each sample for future examination and only rear the remaining half. Actually all the larvae obtained in the first week were preserved, all those caught in the last week were put to breed, while on the remaining occasions about half were preserved and half put to breed. In all 145 larvae were put to breed.

The larvae before being placed on the surface of the soil in these pots were washed in water to remove any of the orthodichlorobenzene emulsion. Very quickly they burrowed into the soil. A muslin cage was put over each pot and the pots were kept moist in an outdoor insectary.

Seventy-four *T. paludosa* and one *T. pagana* Meig.¹ emerged giving a 51 per cent emergence of the larvae originally put in. It was known that a good many larvae escaped from the cages through the hole at the bottom of the flower pot. Therefore the pots were examined in November to ascertain how many larvae had died. Only three dead pupae and one dead larva were found. It is obvious that even allowing for larval cannibalism which probably took place, far more than 51 per cent of the larvae remaining in the pots successfully emerged.

IV. THE HANDLING OF THE ADULT CRANE-FLIES

These breeding cages were examined at least once a day. As soon as crane-flies emerged, they were taken out of the muslin cages and kept separate in glass tubes, 8 by 1½ in., plugged with cotton-wool. Each day these tubes were examined and if it were judged desirable a drop or two of water was allowed to trickle down the inside of the tube. The crane-flies drank this water greedily.

¹ Kindly identified by Dr F. W. Edwards. It is the only British *Tipula* with abbreviated wings in the female and is a common autumn species.

It was found that in no case (out of 74 emergences¹) did emergence take place after 10 a.m. (Greenwich Sun Time). No difference in time was detected in the emergence of the males and females, although it is highly probable that the majority of the males emerge each day before the majority of the females.

The dates of emergence of both sexes, the dates on which the individuals died and the living population for each day are shown in Table II. All the crane-flies emerging were *T. paludosa*, with the solitary exception of one female *T. pagana* that emerged on 9 November. The dates of sampling would to some extent be responsible for this. During sampling for the larvae, empty pupal cases of Tipulids were sometimes noticed protruding from the soil and these were probably those of *T. oleracea* L.

Table II
Rise and fall in numbers of living T. paludosa, 1936 (from
emergence data and longevity trials)

Males				Females			
Date	Emerged	Dead	Population	Date	Emerged	Dead	Population
Aug. 17	1	—	1	Aug. 17	—	—	—
18	—	—	1	18	1	—	1
19	1	—	2	19	—	—	1
20	—	—	2	20	—	—	1
21	—	—	2	21	—	—	1
22	1	—	3	22	—	—	1
23	2	—	5	23	—	1	0
24	1	1	5	24	—	—	0
25	3	2	6	25	2	—	2
26	—	—	6	26	—	—	2
27	3	—	9	27	2	—	4
28	2	—	11	28	1	1	4
29	4	—	15	29	—	1	3
30	5	3	17	30	3	1	5
31	2	2	17	31	8	1	12
Sept. 1	5	1	21	Sept. 1	2	—	14
2	4	4	21	2	1	1	14
3	5	3	23	3	1	3	12
4	1	1	23	4	1	2	11
5	1	2	22	5	3	1	13
6	1	1	22	6	—	1	12
7	—	5	17	7	—	6	6
8	—	3	14	8	2	2	6
9	—	3	11	9	—	2	4
10	—	4	7	10	—	2	2
11	—	2	5	11	—	1	1
12	—	2	3	12	—	1	0
13	—	2	1				
14	—	1	0				

¹ In Table II 42 males and 27 females are dealt with: in addition 2 males were found emerged and eaten by spiders, 2 emerged and later escaped from the glass tubes in which they were being kept, and 1 female was killed immediately it emerged.

Out of 74 emergences, 46 were males and 28 females. Oldham (*loc. cit.*) obtained 7 males and 16 females out of 23 emergences. Pinchin & Anderson (2) reported that of 1292 *T. paludosa* caught in a light trap in 1933 and 1934 at Rothamsted 76.7 per cent were males. The excess of males over females in this latter instance is probably due to the differential response of the two sexes to the attraction of light. Although these workers found that on the whole in the Tipulinae males preponderated in the light trap, in three species, *T. oleracea*, *T. obsoleta* and *T. marmorata*, with which they dealt, the percentage of males caught was lower than that of the females. There is not sufficient data available to determine accurately the proportion of the males and females on the wing.

It will be seen from Table II that the dates of emergence of the males are slightly before those of the females, though the numbers are too low for generalizations to be made. It can, however, be safely postulated that when the living population of crane-flies is at its height, the peak of emergence is past. From the records kept, the average longevity of the males was 7 days (max. 14, min. 1) and that of the females 4-5 days (max. 10, min. 1) (see Table III).

Table III
Longevity of adult T. paludosa in experiment

Number of days alive	Number of males	Number of females
1	1	1
2	1	3
3	4	3
4	5	7
5	5	3
6	2	3
7	5*	4
8	10	1
9	2	1
10	4*	1
11	1	—
12	1	—
13	1	—
14	2	—

* One individual in each of these groups escaped before it died.

When it was desired to pair up individual crane-flies a male was transferred from its own tube to one containing a female. Pairing took place in most cases immediately, but occasionally it was found that a freshly emerged male would not pair. Pairing was generally allowed to take place at about 10 a.m. G.S.T. and lasted for about 2 hours. As soon as it was finished the male was taken out of the tube and the female left

alone. In some instances, no eggs were laid after the first mating so the females in question were allowed to mate again; in others, the females only laid a few eggs after the first mating. Apparently one coition is sufficient to ensure fertilization of all the eggs, but the insects, particularly the males, are not averse to more than one mating.

Table IV
Oviposition of T. paludosa, 1936

Female tag ...	62	74	51	61	71	53	7	5	62	6	50	42	9
Date of emergence	18. viii	25. viii	27. viii	27. viii	28. viii	30. viii	30. viii	30. viii	31. viii	31. viii	31. viii	31. viii	31. viii
19. viii	37	—	—	—	—	—	—	—	—	—	—	—	—
20. viii	144	—	—	—	—	—	—	—	—	—	—	—	—
21. viii	61	—	—	—	—	—	—	—	—	—	—	—	—
22. viii	—	—	—	—	—	—	—	—	—	—	—	—	—
23. viii	—	—	—	—	—	—	—	—	—	—	—	—	—
24. viii	—	—	—	—	—	—	—	—	—	—	—	—	—
25. viii	—	49	—	—	—	—	—	—	—	—	—	—	—
26. viii	—	312	—	—	—	—	—	—	—	—	—	—	—
27. viii	—	—	—	—	—	—	—	—	—	—	—	—	—
28. viii	—	—	240	281	—	—	—	—	—	—	—	—	—
29. viii	—	—	18	—	81	—	—	—	—	—	—	—	—
30. viii	—	—	—	—	92	104	140	219	—	—	—	—	—
31. viii	—	—	—	—	—	35	—	—	232	—	359	216	—
1. ix	—	—	—	—	—	12	—	—	47	—	52	—	260
2. ix	—	—	—	—	—	19	—	—	—	71	—	—	1
3. ix	—	—	—	—	—	—	6	—	—	—	—	—	—
4. ix	—	—	—	—	—	—	—	42	—	—	—	—	49
5. ix	—	—	—	—	—	—	—	—	—	—	—	—	—
6. ix	—	—	—	—	—	—	—	—	—	—	—	—	—
7. ix	—	—	—	—	—	—	—	—	—	121	—	—	—
8. ix	—	—	—	—	—	—	—	—	—	—	—	—	—
9. ix	—	—	—	—	—	—	—	—	—	—	—	—	—
10. ix	—	—	—	—	—	—	—	—	—	—	—	—	—
11. ix	—	—	—	—	—	—	—	—	—	—	—	—	—
	242	361	258	281	173	170	146	261	279	195	411	216	310

Female tag ...	78	39	32	58	61	70	60	80	15	6	37	58	Daily total
Date of emergence	31. viii	1. ix	1. ix	2. ix	5. ix	8. ix	8. ix	25. viii	31. viii	31. viii	5. ix	5. ix	
19. viii	—	—	—	—	—	—	—	—	—	—	—	—	37
20. viii	—	—	—	—	—	—	—	—	—	—	—	—	144
21. viii	—	—	—	—	—	—	—	—	—	—	—	—	61
22. viii	—	—	—	—	—	—	—	—	—	—	—	—	0
23. viii	—	—	—	—	—	—	—	—	—	—	—	—	0
24. viii	—	—	—	—	—	—	—	—	—	—	—	—	0
25. viii	—	—	—	—	—	—	—	49	—	—	—	—	98
26. viii	—	—	—	—	—	—	—	6	—	—	—	—	318
27. viii	—	—	—	—	—	—	—	—	—	—	—	—	0
28. viii	—	—	—	—	—	—	—	—	—	—	—	—	521
29. viii	—	—	—	—	—	—	—	—	—	—	—	—	99
30. viii	—	—	—	—	—	—	—	—	—	—	—	—	555
31. viii	81	—	—	—	—	—	—	—	—	—	—	—	923
1. ix	—	283	—	—	—	—	—	—	—	—	—	—	654
2. ix	—	51	396	182	—	—	—	—	—	—	—	—	723
3. ix	—	—	1	—	—	—	—	—	—	40	—	—	47
4. ix	—	—	—	—	—	—	—	—	—	—	—	—	91
5. ix	—	—	—	78	373	—	—	—	—	—	—	12	463
6. ix	—	—	—	—	56	—	—	—	—	—	—	—	56
7. ix	—	—	—	—	—	—	—	—	40	7	—	—	168
8. ix	—	—	—	—	—	127	—	—	—	—	—	—	127
9. ix	—	—	—	—	—	—	307	—	—	—	12	—	319
10. ix	—	—	—	—	—	—	—	—	—	—	—	—	0
11. ix	—	—	4	—	—	—	—	—	—	—	—	—	4
	81	334	401	260	429	127	307	55	40	47	12	12	5408

The tubes containing the impregnated females were kept vertical with the wad of cotton-wool at the bottom and the curved end of the tube at the top. From 3 to 4 p.m. G.S.T. the females usually started ovipositing and were active throughout the evening. It will be seen later that oviposition took place freely in these tubes with a cotton-wool plug and occasional moistening with water. Rennie(3) stated that in the absence of soil he obtained no eggs.

V. THE CARE OF THE EGGS

The eggs laid up to 7 p.m. G.S.T. were taken out and counted each day. The oviposition of 25 females is given in Table IV in which the date of emergence of each female is shown as well as the number of eggs laid each day and the total number of eggs laid by each. The last five females in the table laid some eggs but in no case did any larvae hatch subsequently. In the first 20 cases at least some hatching took place.

Table V shows the number of eggs and the percentage of the total eggs laid by all the females (25) on the actual day of emergence and each subsequent day. It will be seen that about 45 per cent were laid the day the female emerged. As regular observations ceased each evening at 7 p.m. G.S.T. and egg-laying went on all the evening up till 10 p.m. G.S.T. at least, this figure for the percentage of eggs laid on the day of emergence is too low. It is estimated that 75 per cent of the total eggs are laid by midnight on the actual day of emergence, leaving about 10 per cent for the second day instead of about 40 per cent shown in Table V.

Table V

Period of oviposition, T. paludosa, 1936

	Number of eggs laid	Percentage of total
Day of emergence	2426	44.9
1st day after	2161	39.9
2nd "	342	6.3
3rd "	198	3.6
4th "	67	1.2
5th "	42	0.8
6th "	0	0
7th "	168	3.2
8th "	0	0
9th "	0	0
10th "	4	0.1

Each female was kept till it died and then pickled in 70 per cent alcohol and glycerine for future dissection to find out what proportion of her eggs had been laid. The results of these dissections are shown in Table VI. From this table it can be seen, that excluding the female that

was killed, about 75 per cent of the possible eggs were laid, in 13 cases 100 per cent, in 3 cases between 96 and 99 per cent, in 4 cases between 64 and 76 per cent, and in 7 cases 26 per cent or below.

This figure of 75 per cent of the possible eggs being laid is quite satisfactory and probably is higher than would obtain in a state of nature.

Table VI
Percentage of eggs laid by the females

Female	Date 1936	Eggs laid	Eggs unlaid (by dissection)	Eggs laid %	Total eggs
62	18. viii	242	0	100	242
78	25. viii	Killed	423	0	423
74	25. viii	361	0	100	361
80	25. viii	55	153	26	208
51	27. viii	258	0	100	258
61	27. viii	281	2	99	283
71	28. viii	173	150	54	323
53	30. viii	170	0	100	170
7	30. viii	146	48	75	194
5	30. viii	261	82	76	343
15	31. viii	40	447	8	487
62	31. viii	279	0	100	279
6	31. viii	195	41	83	236
50	31. viii	411	0	100	411
42	31. viii	216	0	100	216
9	31. viii	310	0	100	310
78	31. viii	81	257	24	338
6	31. viii	47	1	99	48
39	1. ix.	334	0	100	334
32	1. ix.	401	18	96	419
58	2. ix.	260	0	100	260
62	3. ix.	0	176	0	176
47	4. ix.	0	272	0	272
37	5. ix.	12	65	15	77
58	5. ix.	12	112	10	124
61	5. ix.	429	0	100	429
70	8. ix.	127	0	100	127
60	8. ix.	307	0	100	307

The actual numbers of eggs laid per female can be seen by consulting Tables IV and VI. In Table VII is shown the frequency of the number of eggs laid as well as that of the total number of eggs per female. In 2 cases no eggs were laid, in 4 cases under 50 and in 2 cases over 50 and under 100. Including all the 27 females allowed to oviposit, the average number of eggs laid was 200; if those 8 females (probably slightly malformed or ill-treated) which laid under 100 eggs are ignored the average is 272. This latter figure is considered nearer the true average. The average of the total number of eggs per female seems to lie between 250 and 350, the range being from 48 to 487.

Table VII

*Number of eggs laid by T. paludosa, 1936 and total
number of eggs per female*

Number of eggs	Frequency of number of eggs laid	Frequency of total number of eggs per female
0	2	0
1-50	4	1
51-100	2	1
101-150	2	2
151-200	3	3
201-250	2	4
251-300	5	5
301-350	3	6
351-400	1	1
401-450	3	4
451-500	—	1

The daily total of eggs obtained from 25 females can be seen on reference to Table IV (last column). These figures can be taken as a sample population of freshly laid eggs. By comparing these figures with the dates of emergence of the adult females (Table II) it is seen that on 31 August most eggs were laid, while the crest of emergence of the females occurred on 31 August but most females were alive on 1 and 2 September. In other words, since the females live for some days after oviposition, it is highly probable that, when the greatest numbers of females (and both sexes as well) are seen on the wing, the majority of the eggs (i.e. peak of oviposition) has already been laid and also that the peak number of eggs in the soil will have occurred. Only approximate figures for the population of eggs from day to day are available as 100 per cent larval hatching did not take place and it was impossible to separate fertile from unfertile eggs.

On removing the eggs each evening from the oviposition tubes they were placed after counting in solid watch-glasses (one per parent crane-fly) and kept moist by putting a drop or two of water actually on the eggs. The watch-glasses were placed in culture dishes in which there was water, the data being written in indian ink on the covers of these culture dishes. Thus the eggs were primarily kept moist by the water on them and, if this happened to dry unnoticed, the whole atmosphere inside the culture dish was still comparatively moist. These egg-containers were kept on a shelf in the laboratory at room temperature (August to September). Mould in some cases crept in but did not become serious until after the larvae had finished hatching.

Table VIII
Hatching of T. paludosa larvae, 1936

Female Date of emergence	...	62 18. viii	74 25. viii	51 27. viii	61 28. viii	71 30. viii	53 30. viii	7 30. viii	5 31. viii	62 31. viii	6 31. viii	50 31. viii	42 31. viii	9 31. viii	78 31. viii	39 1. ix	32 1. ix	58 2. ix	61 5. ix	70 8. ix	60 8. ix	Daily total
Date of larvae hatching																						
31. viii	11	56	1. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11
36	36	2. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	56
11	11	3. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	36
4. ix	2	4. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11
5. ix	3	5. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
6. ix	—	6. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
7. ix	—	7. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
8. ix	—	8. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	28
9. ix	—	34	8. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	34
10. ix	—	58	15	4	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	87
11. ix	—	47	4	2	3	38	2	43	8	—	—	—	—	—	—	—	—	—	—	—	—	107
12. ix	—	27	2	1	25	31	30	87	14	8	—	—	—	—	—	—	—	—	—	—	—	142
13. ix	—	14	1	1	27	23	40	51	14	14	—	—	—	—	—	—	—	—	—	—	—	218
14. ix	—	12	2	1	10	9	24	2	19	17	69	30	8	6	1	27	3	1	—	—	—	230
15. ix	—	4	—	—	9	5	5	3	25	33	62	15	4	4	11	45	4	9	—	—	—	192
16. ix	—	1	—	—	6	6	1	1	14	12	33	7	22	9	12	8	4	23	—	—	—	261
17. ix	—	1	—	—	1	1	1	1	4	3	6	5	11	7	10	5	8	24	—	—	—	233
18. ix	—	—	—	—	3	—	—	—	2	1	3	16	7	5	13	—	1	27	—	—	—	140
19. ix	—	1	—	—	20	ix	—	—	5	—	—	29	6	5	7	19	6	17	12	2	—	72
20. ix	—	—	—	—	—	—	—	—	—	—	—	6	5	5	7	19	1	7	17	—	—	80
21. ix	—	—	—	—	—	—	—	—	5	—	—	7	6	6	1	9	1	6	14	40	—	103
22. ix	—	—	—	—	—	—	—	—	—	—	—	3	4	4	2	12	—	11	15	34	7	58
23. ix	—	—	—	—	—	—	—	—	2	—	—	1	1	3	2	13	—	6	5	15	13	92
24. ix	—	—	—	—	—	—	—	—	—	—	—	3	1	1	—	1	—	11	15	34	13	44
25. ix	—	—	—	—	—	—	—	—	1	—	—	1	—	—	—	3	—	9	4	2	10	30
26. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	1	3	2	1	14	24
27. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	30	35
28. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17	12
29. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9	9
30. ix	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	5
1. x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	3
2. x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	3
3. x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	7
4. x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	8
5. x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9	9
6. x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Total larvae	119	287	44	23	88	128	128	208	99	68	303	152	74	50	233	15	162	70	111	136	136	2498
% hatch	49.2	79.5	17.1	8.2	50.8	87.7	87.7	79.6	35.5	34.8	73.7	70.4	23.9	61.7	69.8	3.7	62.3	16.3	87.4	44.3	44.3	

VI. THE REARING OF THE EARLY LARVAL INSTARS

The larvae started to hatch in all 20 cases between 11 and 15 days after the emergence of the parent female crane-fly (twice on the 11th day, 5 times on the 12th, 8 times on the 13th, 4 times on the 14th and once on the 15th day after). Full details of the hatching can be seen by referring to Table VIII in which the numbers of larvae hatching daily can be seen and in the last column the total numbers hatching each day. The peak of larval hatching took place about 12-16 September. The percentage hatches are also shown in this table. The frequency is shown in Table IX and, considering the method of keeping the eggs, these figures are fairly satisfactory. Roughly 46 per cent of the eggs obtained developed into larvae, 2498 larvae from 5408 eggs.

Table IX

Frequency of percentage larval hatch, T. paludosa, 1936

Percentage hatch	Frequency
0	5
1- 10	2
11- 20	2
21- 30	1
31- 40	2
41- 50	2
51- 60	1
61- 70	4
71- 80	4
81- 90	2
91-100	0

Each day the larvae which had hatched were taken from the watch-glasses and placed in Petri dishes, one for a single female progeny. These were kept moist and a variety of foods for the larvae were used. In the first instance a single germinating corn of wheat per Petri dish was used (Pl. XXVII). The larvae fed on the roots and root hairs quite satisfactorily. The advantage of this food is that the head-capsules of the young larvae after ecdysis are easily seen. Chickweed, clover and pieces of cabbage leaves were also used with success. Slices of potatoes proved satisfactory as also did ordinary bran. Finally bran was chosen as the staple food and the head-capsules were floated out occasionally.

Rapid growth took place and the first two instars were passed in roughly 14 days. As a check on the mortality of the larvae under these conditions, 11 Petri dishes were examined on 1 and 2 October in detail. Roughly 46 per cent of the larvae put in were recovered after this lapse of time as will be seen from Table X. It will be noted that the percentage recovery does not bear a direct relationship to the numbers of larvae

originally put in the Petri dishes and that it varies from 7 to 87 per cent. It is known that the mortality is partly due to two factors, the first being escaping larvae, the second cannibalism. The latter was very severe and, as soon as a larva was in rather poor condition for any reason, healthy ones attacked it and soon killed it. This was observed frequently. The problem of larvae escaping revealed an interesting fact. The lids of the Petri dishes did not fit close enough to prevent larvae getting out, but these larvae were always found dead and shrivelled on the white paper placed under each Petri dish. This paper was originally used to facilitate seeing the larvae, but it was more useful in catching the escaping larvae. Apparently the escaping larvae could not withstand the dry conditions outside the breeding cages and succumbed before they could move more than a few inches. This great susceptibility to drought in the first two instars may be largely responsible for the common statement that mild wet Augusts and Septembers are advantageous to leatherjackets. The larvae can also be recovered as they escape by placing the Petri dish on a tripod standing in a dish of water.

Table X

Recovery of larvae from Petri dish breeding cages, 1-2. x. 36 (arranged in descending order of numbers of larvae originally put in)

Female	Larvae put in	Larvae recovered alive	Percentage recovery
50	302	161	53
39	228	91	40
58	161	51	32
42	151	89	59
62	98	40	41
71	88	24	27
9	74	33	45
6	68	59	87
78	49	14	29
61	23	13	57
32	15	1	7

The larvae in the 3rd instar were fed satisfactorily on bran with pieces of cabbage leaves and seemed quite healthy. The experiment stopped at this stage.

VII. SUMMARY

1. Full-grown larvae of *Tipula paludosa* Meigen were obtained by application to the soil of an emulsion of orthodichlorobenzene, and it is suggested that the larvae become non-susceptible to this treatment about 6 weeks before pupation. The larvae were reared in pots of soil containing germinating and growing wheat.

2. The adult crane-flies were kept in glass tubes for longevity trials which showed that on the average the males lived about 7 and the females 4-5 days. Mating and oviposition took place in these tubes.

3. Twenty-seven females laid 5408 eggs, averaging 200 each. About 45 per cent of the eggs were laid the day the female emerged and 40 per cent the first day after. It is believed that these figures are too low as the regular observations stopped at 7 p.m. G.S.T. and it is suggested that 75 per cent of the eggs are in reality laid by midnight on the actual day of emergence and 10 per cent on the first day after. The eggs were kept damp in solid watch-glasses which were stood in water in culture dishes.

4. It is shown that by the time the peak of crane-flies on the wing has been reached, the peak of eggs in the soil will already have been passed.

5. Hatching took place 11-15 days after the emergence of the parent crane-fly, 2498 hatching from 5408 eggs. The young larvae were kept in Petri dishes and fed on wheat rootlets, clover or chickweed leaves, pieces of cabbage leaves, slices of potato or bran. By the time the 3rd instar was reached about 54 per cent mortality had occurred, chiefly owing to larvae escaping and cannibalism (576 survived out of 1257). This stage was reached in roughly 14 days.

6. The breeding potential was as follows: 51 per cent of the nearly full-grown larvae emerged as adults (this figure is low because of the number of larvae escaping); 75 per cent of the available eggs were laid by the females; 46 per cent of the eggs laid hatched, and 46 per cent of the larvae survived the first two instars. In other words, under the conditions of the experiment, on the average, one female crane-fly laid 200 eggs, 92 larvae hatched and 44 survived the first two instars.

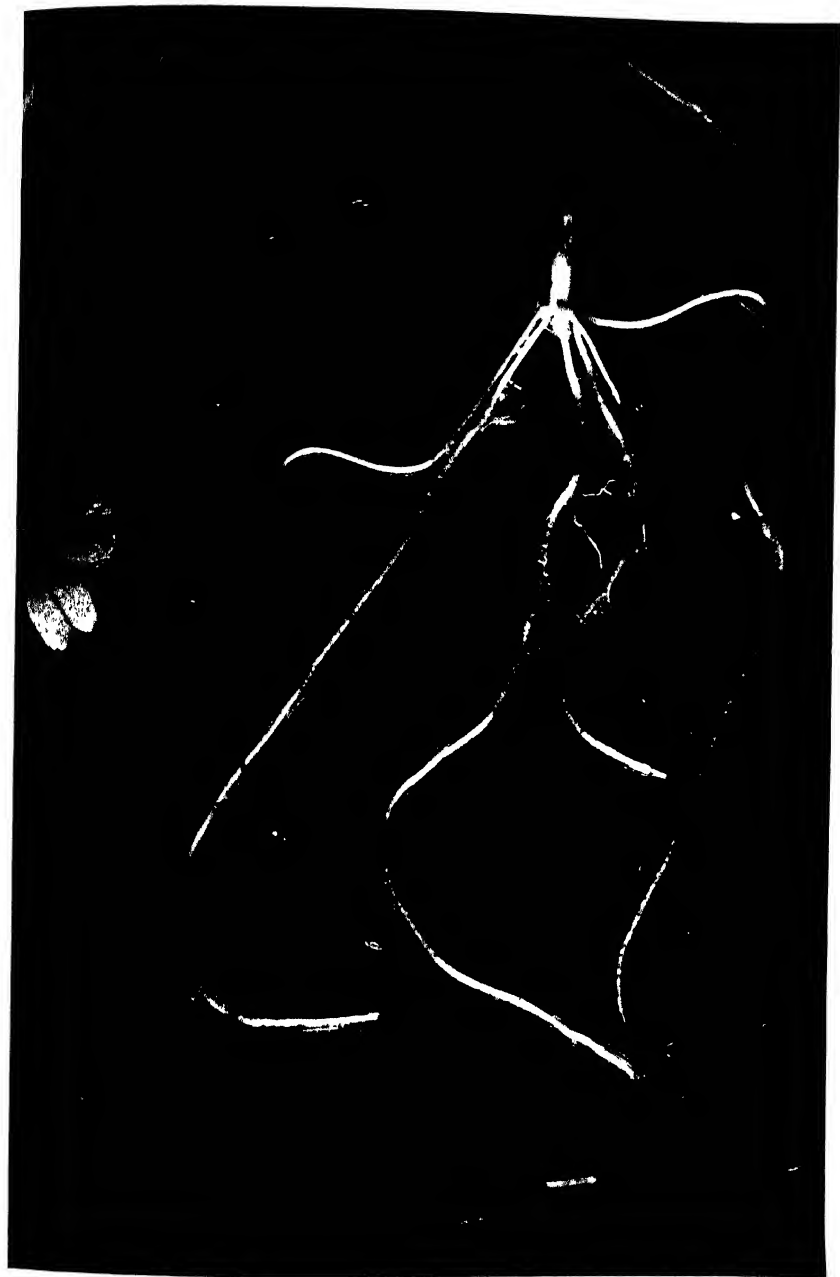
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EXPLANATION OF PLATE XXVII

Method of rearing larvae of *T. paludosa* on wheat rootlets in Petri dish.

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BARNES.—METHODS OF INVESTIGATING THE BIONOMICS OF THE COMMON CRANE-FLY, *TIPULA PALUDOSA* MEIGEN, TOGETHER WITH SOME RESULTS (pp. 356-368)

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THE ASPARAGUS MINER (*MELANAGROMYZA SIMPLEX* H. LOEW) (AGROMYZIDAE; DIPTERA)

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(With Plates XXXVI and XXXVII and 1 Text-figure)

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I. INTRODUCTION AND METHODS

THE following paper deals with the morphology and bionomics of the asparagus miner, *Melanagromyza simplex* H. Loew as studied at Harpenden, Herts. This is an agromyzid fly whose larvae mine in the stems of asparagus. It must not be confused with the asparagus fly, *Platyparea poeciloptera* Schrank, a trypetid whose larvae bore in the stems and are a serious pest on the continent of Europe.

I am indebted to Dr C. L. Walton of the Long Ashton Research Station for drawing my attention in the first instance to the miner, following the receipt by him of puparia from the Evesham asparagus-

growing area. A short joint note concerning the insect was written in 1934⁽¹⁾ and since that time the present writer has been in sole charge of the investigation. Mr J. E. Collin has kindly identified the miners and Dr Ch. Ferrière has similarly identified the parasites. Sir John Russell, the Director of Rothamsted Experimental Station, has allowed free access at all times to his asparagus beds and without this the work could not have been done. Mr V. Stansfield is responsible for the photographs, while Dr F. M. L. Sheffield took the photomicrographs of the eggs and larvae.

The part of the paper relating to the bionomics of the miner consists essentially of an observational field study supported by a few insectary breeding experiments. Usually, investigations of this nature tend to consist of insectary experiments supported by a few field observations. In the present instance beds of asparagus in the grounds of Laboratory House, Harpenden, were kept under close observation and fresh material was collected periodically. All the main processes of life, such as mating, oviposition and growth were watched out-of-doors.

Ordinary breeding apparatus consisting of pots and muslin or cellophane cages were used for the insectary side of the investigation.

The eggs were mounted in De Faure's fluid, while larvae were mounted in several ways: in De Faure's fluid, glycerine jelly, euparal and in balsam. The usual methods of clearing by potash and of staining by carbol-fuchsin were also used.

II. HISTORY AND IDENTIFICATION

The agromyzid in question was first discovered and described in 1869 by H. Loew⁽¹⁶⁾ occurring in Pennsylvania, New Jersey and New York in North America. It was not, however, until 1898 that its host plant was suggested by Chittenden⁽²⁾ to be asparagus. He had caught the flies on asparagus plants in 1897 at Cabin John, Maryland. Previously, however, Sirrine⁽²⁰⁾ had found the puparia under the epidermis of asparagus in 1896 and reared the flies, thus partially working out the life history. He gave it the name "asparagus miner". In 1907 Chittenden⁽³⁾ issued a bulletin in which he traced the distribution of this miner in the United States of America and the same author's circular⁽¹⁾ is a revised reprint of this bulletin. The most comprehensive study of the insect in America is that by Fink⁽¹¹⁾. Numerous United States records are cited in this bulletin and Fink stated that at that time the miner was widely distributed wherever asparagus was grown in the eastern part of the United States including the States of New York, New Jersey,

Pennsylvania, Maryland, Connecticut, Massachusetts, Long Island, District of Columbia, Tennessee, Virginia and California. This study of the miner remains the most useful reference work and the illustrations of all stages are good, particularly those of the adult flies. Drake & Harris (10), later mentioned it as occurring in Iowa.

In Europe, the insect was discovered as a pest of asparagus at much the same date as in America. Sajo (18, 19) found it in central Hungary but identified it as *Melanagromyza maura* Meigen. This was in error as *maura* Meigen is a distinct species from *simplex* H. Loew and its host-plants are various species of *Solidago* and *Aster amellus* according to Hendel (13) in Lindner (1931). Giard (12) then recorded *Melanagromyza simplex* from France in 1904, while Lesne (15) gave further notes on its habits in France. Collin (5) next reported it in England in 1911. Soraue (21) also mentioned it in his *Handbuch* in 1913 and uses figures from Chittenden, though in a later edition (1928) (22), on pp. 29 and 30, *M. simplex* was confused with *Platyparea poeciloptera* and actually Fink's figures of *simplex* were used to illustrate the remarks on *poeciloptera*. It was then stated that the latter fly is found in North America and had two generations a year there. This is incorrect. In dealing with *Melanagromyza simplex* the letterpress and figures were, on the other hand, correct. Dingler (6, 7, 8, 9) dealt with *M. simplex* as an insect of asparagus in Germany, but stated that, at present, it cannot be considered to be a pest as it is rare.

The synonymy of *M. simplex* is as follows:

simplex H. Loew (1869) = *maura* Sajo (1896) (nec *maura* Meigen).

III. MORPHOLOGY

(a) The egg

The egg (Pl. XXXVI, fig. 1) is broadly cylindrical, slightly flattened at the micropylar end and slightly pointed at the other. It is between 2 and $2\frac{1}{2}$ times as long as broad and measures about 0.48 mm. in length and about 0.22 mm. at its widest point. No sculpturing nor reticulation has been observed on the chorion. The micropyle is obvious at the more blunt end of the egg which is placed farthest in the tissue of the plant, the pointed end being towards the surface. The egg is entirely inserted in the plant tissue, the long axis of the egg being about 45° to the horizontal.

(b) The larva

1st instar. The newly hatched larva (Pl. XXXVI, fig. 2) is of peculiar shape and appears to be metapneustic. The total length of the larva is about 0.4 mm. when taken out of the plant tissue and so relieved of the pressure which causes an elongation to take place.

The cephalo-pharyngeal skeleton is very prominent and brownish yellow in colour. Its total length is about 0.2 mm. or about half the length of the whole larva on hatching. On the mandibular sclerites, the actual mouth-hooks have a slightly serrated appearance. At the bases of the latter there is a darker rounded portion, while the posterior ends of these sclerites, which may be fused accessory sclerites, are also heavily chitinized. There is a trace of the dentate sclerite. The pharyngeal sclerite, completely fused with the intermediate or hypostomal sclerite, is long in proportion to larval length on comparison with those in the latter instars. Its anterior process, including the intermediate sclerite, is about two-thirds the length of the whole sclerite. The dorsal processes are slightly longer than the ventral process and are slightly broader than the anterior process. The main chitinized portion of the ventral process is considerably narrower than the anterior process, but chitinization has only taken place in a narrow strip along its basal margin and to a less extent in a separate median and dorsal area.

2nd instar. The second instar larva (Pl. XXXVI, fig. 3) is about 2 mm. long. The shape is elongate, the larva being about four times as long as broad. The calcospherites, typical of agromyzid larvae in general, are now conspicuous in the fat bodies and are clearly visible in the illustration. These bodies start forming in the later part of the 1st instar.

The cephalo-pharyngeal skeleton is about 0.4 mm. long. It is now much more heavily chitinized and almost black in colour. The mouth-hook has a large apical tooth and two smaller ones of about equal size, although the middle tooth shows indications of stronger development. The ventral process of the pharyngeal sclerite is less heavily chitinized than the rest, but decidedly more so than in the 1st instar. The proportions of the lengths of the anterior process and the whole sclerite are now reduced, the anterior process being slightly less than half, whereas in the previous instar it is about two-thirds. Otherwise there is not much difference in the cephalo-pharyngeal skeleton of the first two instars.

The larva is now amphipneustic and remains so for the rest of its life. Both the anterior and posterior spiracles are stalked.

3rd instar. The 3rd and last larval instar (Pl. XXXVI, fig. 4) is about 3-5 mm. long and of about the same proportions as the 2nd instar. The fat bodies are crammed with a large number of calcospherites.

The cephalo-pharyngeal skeleton is now nearly 0.9 mm. in length and black through heavy chitinization. The mouth-hook has a large and sharp apical tooth and a middle tooth which is also pointed and decidedly larger than the basal one which is very small in comparison. In this instar the anterior process of the pharyngeal sclerite has become differentiated as the intermediate sclerite which is blunt-ended at both ends, broader distally and completely separated from the pharyngeal sclerite. On the latter there are no lateral processes. The intermediate sclerite (in the two previous instars the anterior process of the pharyngeal sclerite) is distinctly less than half the length of the intermediate and pharyngeal sclerites. There is no anterior process of the pharyngeal sclerite.

It has been suggested to the writer that the posterior spiracles in this stage are reminiscent of the polypneustic form which occurs in the Hippoboscidae. Both pairs are stalked and are very prominent in this last instar. A detailed study of them as well as of the spiracles in the other instars would be of interest.

In this instar a band of outicular processes is prominent on each segment ventrally; in the previous instar they are not nearly so obvious. Each of these bands on the abdominal segments consists anteriorly of a row or two of large tooth-like processes pointing forwards, then a middle area covered with small processes pointing upwards, and posteriorly a row or two of large tooth-like processes pointing backwards. The exact number of rows seems to vary on the different segments. On the prothoracic segment there are rows of fine cuticular processes, the anterior row pointing forward, the remaining ones directed backwards.

Characters for the separation of the three larval instars. The different instars are easily recognized and the most obvious characters used in their separation are shown in Table I. The characters, besides being checked by an examination of many larvae, have been confirmed by finding and mounting larvae in the actual process of moulting.

Table I

Characters for the separation of the three larval instars

	1st instar	2nd instar	3rd instar
Anterior spiracles	Absent	Present	Present
Mouth-hook	Serrate	One large and two small teeth	One large, one medium and one insignificant tooth
Length of anterior process of pharyngeal sclerite compared with total length of pharyngeal sclerite	Two-thirds	Less than half	Nil
Intermediate sclerite	Fused as anterior process of pharyngeal sclerite	Fused as anterior process of pharyngeal sclerite	Differentiated and separate

(c) The puparium

The puparium (Pl. XXXVII, figs. 1-3) is light to dark brown in colour and about 4 mm. in length; it is highly reminiscent of the "flax seed" of the Hessian fly. It is flattened dorso-ventrally. At each end is a pair of hooks by means of which it remains attached to the asparagus stem even when the latter is firmly shaken.

(d) The adult fly

The adult fly (Pl. XXXVII, figs. 4-6) is a small, exceedingly shiny and entirely black insect, $2\frac{1}{2}$ - $3\frac{1}{2}$ mm. (nearly $\frac{1}{8}$ of an inch) in body length. The female has a rounded abdomen and takes up a characteristic position of rest in which the wings are placed flat across the body with their posterior margins just separated. Fink(11) (Fig. 127) illustrated this well. In Pl. XXXVII, figs. 4 and 6 the abdomen has shrunk after death. The wings are glistening. Fink(11) stated that one of the striking features in the adult was the picture of a face on the thorax but the present investigator has not seen this.

IV. DISTRIBUTION

Apparently the first record of this fly's occurrence in England is that of Collin⁽⁵⁾ in 1911, who stated that he had taken it only by sweeping over asparagus beds in Suffolk in July and early August. The next published record was that of Barnes & Walton⁽¹⁾ who reported its occurrence in the Evesham asparagus area and in a garden at Harpenden. They also recorded that Mr A. H. Hamm had captured it at Oxford in 1917 and, in addition, that Dr H. W. Miles had sent asparagus stems containing puparia from Formby, Lancs, and Mr G. Fox Wilson had found puparia at Wisley, Surrey. Since then the present writer has found puparia in asparagus stems sent during 1934 from Godmersham, Kent (H. F. B.); Chickerell, Dorset (C. S. F.); Downham, Norfolk (E. R. P.); Williton, Somerset (J. R.); Marlborough, Wilts (G. P.); and Nottinghamshire (A. R.). It would thus appear reasonable to suppose that the miner is generally distributed throughout this country.

Abroad, it has been recorded from Hungary, France, Germany and the United States of America. Its comparative unimportance as a pest has probably precluded it from being reported in other countries. For more details as to its distribution abroad reference should be made to the section of this paper dealing with its history.

V. BIONOMICS

(a) Emergence, mating and oviposition

Emergence can easily be made to take place during the winter months by keeping the puparia in a warm room. For example, puparia collected during September 1933 at Harpenden were placed on sand and kept in the laboratory. The flies started emerging on 23 December and continued spasmodically until 1 August 1934. This long period of emergence was probably due to the careless way in which the puparia were kept, especially as regards moisture. During February the flies lived indoors for from 7 to 9 days when fed on sugar solution.

From material collected at the same time but kept in an outdoor unheated insectary the flies started emerging on 9 June 1934 and continued until 16 July. From material collected on 2 July, flies of the second flight emerged between 24 August and 2 September.

Out-of-doors at Harpenden in 1934 the flies were seen first on 4 June, with the largest numbers on 10 June and the last on 28 July. In 1935 the equivalent dates were 9 and 29 June and 30 July. These dates refer to the first flight of flies only.

The second flight of flies in 1934 started on 5 August and the last was seen on 13 September. In 1935 this flight started on 15 August.

Mating was first seen out-of-doors in 1934 on 9 June and on 21 June in 1935. Other dates on which mating was observed in 1934 were 10, 14, 15, 18, 21, 23 and 30 June, 1, 4, 5, 10 and 22 July. Among the second generation mating was observed on 5 and 18 August. Other dates in 1935 were 24, 26 and 30 June, 3, 5, 9, 11, 22, 24 and 29 July and 17 August. This last date concerned flies of the second generation, while all the other dates relate to the first generation. In 1934 mating was first noted 5 days after the first fly was seen; in 1935 mating was not seen until 13 days after the appearance of the flies. Fink⁽¹¹⁾ stated that copulation usually begins 2-3 days after the females appear, the males appearing several days earlier. In 1912, a late cold season, the flies started emerging near Ithaca, New York, on 26 May. This date can be compared with 4 June in 1934 and 9 June in 1935, the dates on which the flies were first seen at Harpenden.

Mating in the insectary was observed on 10, 11 and 14 June in 1934. Coition was seen on one occasion to start at 9.30 a.m. and continue until 11.45 a.m., on another occasion it started about the same time and continued until 12.45 p.m. Out-of-doors flies were usually seen mating during the morning and on one occasion at 7.45 p.m.

Fink went on to state that oviposition begins 1-2 days after mating. Observations at Harpenden showed that oviposition took place on 12, 15 and 26 June in 1934 out-of-doors and in the insectary on 10 and 11 June. It must be understood that these dates only refer to actual observations and no suggestion is made that mating, oviposition, etc. only took place on these days. Oviposition was seen to take place exactly in the manner Fink described and it may be useful to quote his words. "Before beginning to oviposit the female is usually seen running up and down an asparagus stalk, searching with her proboscis for a suitable place in which to lay her eggs. After this preliminary test of the entire stalk, the female usually begins to oviposit at the base of the stalk at or near the surface of the soil; sometimes, according to the writer's observations, if the soil around the base of the stalk is loose the female even ventures below the surface as far as possible. She then takes up a position with her body parallel to the long axis of the stalk, and, bending her abdomen at almost a right angle to the stalk, begins to work her ovipositor into the epidermis. The ovipositor eventually pierces the epidermis and the egg is inserted just beneath it. The process of probing the stalk is

repeated after each egg is deposited." All the points mentioned in this quotation have been observed by the present writer.

The eggs when laid are extremely difficult to find unless the female is watched and then the epidermis almost immediately peeled off at the puncture mark. The eggs are completely inserted in the stem (Pl. XXXVI, fig. 1). At first white, the egg soon becomes almost transparent and apparently the same colour as the tissues of the asparagus.

Oviposition has usually been observed on the flowering and shooting stalks and only occasionally on the cutting stems. No seed bed has been under observation, but Fink states that seedlings offer a place for the flies to oviposit.

(b) *Life cycle*

As has already been stated, the flies start emerging at the beginning of June and continue until the end of July. Mating takes place a few days after emergence, oviposition following quite quickly. There is a long period of emergence and consequently also of mating and oviposition. No exact figures can be given for the duration of the egg stage but larvae were first found out-of-doors in 1934 on 28 June, this being 17 days after first finding the eggs. Fink stated that the incubation period is approximately 12-18 days. The larvae at once begin to mine in a zigzag fashion up the stalk and continue for some distance, as much sometimes as 12 in. Then they turn downwards and finally reach about ground level when they are full grown and ready to pupate. Often, however, in this first or summer generation the larvae have not again approached soil level by this time. Consequently puparia are found at any height on the stalks (Pl. XXXVII, figs. 2-3). The mines are often by no means as straight or uniform as shown in the figure. In 1934 larvae in the 2nd instar were first found on 2 July, the 3rd instar first on 4 July and the first puparium on 7 July. In this year flies of the second generation were first seen on 5 August. It would thus appear that the egg stage takes about 2 or 3 weeks, the larval stages are short and that the pupal stage lasts about a month. This agrees well with what Fink observed. In his case the larvae hatched about the middle of June, they attained full growth by the end of June to the first week in July, pupation commenced on 3 July and flies began to emerge on 20 July.

The flies resulting from this first generation are on the wing from the beginning of August until about the second week in September (see Text-fig. 1). The life history of the second generation is very similar to that of the first except that the puparia are usually found just above or

below soil level (about 5 in.) and not high up the stalks. Fink found the depth below the surface varied from $\frac{1}{2}$ to 7 in. The period of pupation is much longer, the puparia over-wintering and the flies not emerging until the following spring.

The second generation is not a complete one and many of the puparia of the first generation over-winter. In New York there are likewise two generations in the year and there the larvae of the second attain full growth by about the end of August or the beginning of September. In this country this point in development must be reached later and perhaps accounts for the second generation being only partial.

The adult flies are extremely active during sunshine but will remain for long periods in the same position during cold sunless intervals. At nightfall they take up definite positions where they remain until the following morning. They usually rest among the leaves on the flowering and fruiting stalks.

(c) *Damage*

According to the present observations there is usually very little damage done by the larvae of this miner, at any rate in mature asparagus beds. When several larvae are present, the stems of the flowering stalks certainly become girdled with the mines; but unless there are really large numbers present the plants do not suffer. In the beds under observation several puparia were usually found in each stem but no injury was noticed. As a result of the mines the outer skin of the lower stem and root becomes dry and flakes off (Pl. XXXVII, fig. 7). Chittenden (3, 4), in America, found as many as nine puparia to an inch on the stalks. Fink (11) described the signs of attack as a yellowing of the stalk at the base, a shrivelled appearance and a premature yellowing of the entire plant. Other American writers have commented on the presence of these puparia on the roots and have claimed that this type of attack forces one to consider the miner as a pest. Dingler (8) stated that *M. simplex* could not be regarded as a pest of asparagus in Germany, but might become one.

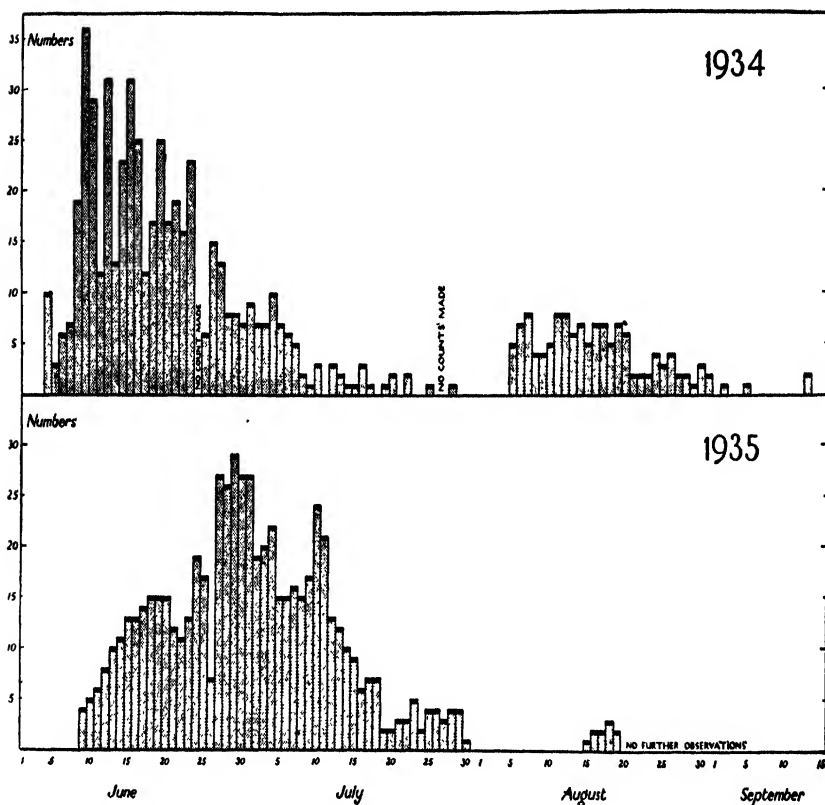
Fink (11) stated that the flies lay eggs on volunteer plants and seeding beds and make no attempt to oviposit on cutting beds. He also states that this instinct not to lay on cutting beds but to concentrate on seedling plants and on plants that are newly set out is the cause of severe early damage. The young plants go yellowish and the stalks shrivel and die. The present writer has not had the opportunity of keeping a seedling bed under observation. The beds in Laboratory House garden are cutting beds and gaps were filled by mature plants from other beds.

In some stems containing the puparia of this miner sent by Dr C. L. Walton there were in addition some other dipterous larvae. These proved on emergence of the flies to be those of *Lonchaea flavidipennis* Zett. (kindly identified by Mr J. E. Collin). Larvae of the same fly were also present in asparagus stalks attacked by *Melanagromyza simplex* at Harpenden. Stems attacked by *M. simplex* appear to be more attractive to this fly than healthy ones. In such cases *M. simplex* must be considered injurious as it attracts the larvae of *Lonchaea flavidipennis* which seriously damage the asparagus stems. Newton⁽¹⁷⁾ reported this species of *Lonchaea* from *Hyoscyamus* stems attacked by flea-beetles.

(d) *Local abundance*

In order to assess the local abundance of the miner the asparagus plants in the Director's garden were examined twice daily and all the flies seen were counted. These counts were made between 9 and 10 a.m. (summer-time) and again between 6 and 8 p.m. each day in 1934 from 1 May until 1 October with the exception of 29 May to 3 June inclusive, 24 June and 26-27 July inclusive. In 1935 the daily observations were made from 1 May till 19 August inclusive. In this year, however, the times were slightly different, from 1 May to 21 June at about 9-10 a.m. and at 8 p.m.; 22 June to 21 July at about 7 a.m. and again at 9 p.m.; 22 July to 19 August between 9 and 10 a.m. and at 7.30 p.m. The object of altering the times at which the observations were made was to enable the flies to be counted while at rest on the asparagus. Towards evening the flies take up their positions of rest on the plants and remain there until the following morning. As this is so, if the evening count be made after the flies have taken up their positions for the night, the morning count serves as a check on the previous evening's. The evening count would include those flies which had emerged that day and survived. In Text-fig. 1 the results of these observations made in 1934 and 1935 are shown. The maximum number of flies seen either in the morning or evening is given for each day. It should be realized that up to 3 July 1934 two beds of asparagus were under observation and since that date only one. The reason for this was that on that date one bed was done away with, some of the plants from it being used to fill gaps in the remaining bed and others being thrown away. The effect of this should not alter the counts very much as the beds were in close proximity and the flies could easily pass from one to the other. But on the other hand some puparia must have been removed with the plants that were lifted

and destroyed. Further in 1934 some flies (88 in all) were removed from the asparagus beds to breeding cages for experimental purposes. Thus on 4 June 10 flies were removed, on the 5th 3, on the 6th 6, on the 7th 6, on the 8th 17, on the 9th 15, on the 11th 7, on the 12th 20 and on the 15th 4. After this date no further flies were taken away. The net result of this removal of plants and flies is to make the curve of 1934 in Text-fig. 1 err on the low side.



Text-fig. 1. Abundance of *Melanagromyza simplex* adults observed in 1934 and 1935.

However, the curves of abundance show when the flies first appeared, when the crest in numbers was reached, when the first flight was over and when the second flight occurred. They also give some idea of the abundance of the flies although this can only be approximately accurate since the duration of life of the individuals was not traced. These curves also resemble what might be expected of actual emergence curves.

VI. PARASITES

Giard (12) described *Dacnusa rondanii* as parasitic on this fly, but so far as the writer is aware no other parasites have been recorded until the undermentioned parasites were noted by Barnes & Walton (1).

Three parasites, kindly identified by Dr Ferrière, have been reared from *Melanagromyza simplex* material collected at Harpenden. They are as follows: a braconid, *Dacnusa bathyzona* Marsh.; a pteromalid, *Sphegigaster* sp.; and a eulophid, *Pleurotropis epigonus* Walk. The braconid and eulophid have also been reared from material collected in the Evesham area. As with the fly, the parasites can be induced to emerge indoors during the winter. Another point of similarity is that *Dacnusa bathyzona* does not always emerge from the puparia of the first generation of *Melanagromyza simplex* the same year. One example of this was shown by a puparium of *M. simplex* found on 7 July 1934 which was parasitized by this species and the parasite did not emerge until 10 June 1935. In this case the parasite lived for 7 days. This delayed emergence may be the rule and not the exception in the case of the parasite.

In one breeding cage of over-wintering puparia (1933-4) kept out in the insectary, *Dacnusa bathyzona* emerged between 3 June 1934 and 15 July, *Pleurotropis epigonus* Walk. between 23 May and 15 June and an unidentified parasite emerged on 12 September. The *Melanagromyza simplex* emerged in this cage between 9 June and 16 July. The comparative numbers from this breeding were *M. simplex* 25, *Dacnusa bathyzona* 21, *Pleurotropis epigonus* 8 and 1 unidentified parasite.

In another cage of material collected on 2 July 1934, *Dacnusa bathyzona* emerged between 24 August and 8 September, and unidentified parasites between 25 August and 8 September. The *Melanagromyza* emerged in this case between 24 August and 2 September. The comparative numbers in this instance were *M. simplex* 5, *Dacnusa bathyzona* 6 and 7 unidentified parasites.

It would appear from these examples that parasitism in the over-wintering generation of 1933-4 and the summer one of 1934 was extensive.

Out-of-doors it was possible to make observations on *D. bathyzona* in 1934. This parasite was first seen on 7 June and the last on 8 July. In all it was seen on 14 days within these extremes. The females were on several occasions seen searching for the *Melanagromyza simplex* in the stems of the asparagus. First they hovered round the stems and then, having alighted, ran up and down with wings "cocked" and definitely

"feeling" with their antennae. Just as the flies while ovipositing go right down the cracks in the soil round the plants' stems on occasion, so do the *Dacnusa bathyzona*. No specimens of this parasite were observed in the asparagus beds while the second brood of *Melanagromyza simplex* was on the wing in 1934, although specimens were bred in the insectary from material gathered on 2 July as stated in the preceding paragraph.

VII. CONTROL

Fink⁽¹¹⁾ reviewed the methods of control suggested up to that date. These include Chittenden's method of allowing a few volunteer plants to grow during the cutting season in order that the flies might oviposit on them and then burning the infested plants. Sirrine⁽²⁰⁾ had suggested pulling the stalks of a badly infested bed late in the autumn and thus destroying the puparia. This seems to be the simplest method when asparagus is grown on a small scale. Fink himself experimented with nicotine sprays for killing both the larvae and the flies and found that "Black leaf 40" tobacco extract, in the proportion of 1-500 gal. of water, appeared to be adequate.

Dingler⁽⁹⁾ dealt thoroughly with control measures against the asparagus fly and the asparagus miner and states that indirect methods must be used. When asparagus is grown on a small scale the following are some methods to be employed: traps for catching the ovipositing females, covering the shoots with paper or cellophane and collecting the adult flies in the early morning by placing rods covered with an adhesive among the crop. This last method has been tried by the present writer. He used clothes-pegs smeared with grease banding material but found that, although a few flies were caught, more flies were not. This held true whether the pegs were painted green or not.

When asparagus is grown on a large scale Dingler suggested extending the harvesting time until the end of the flight period, the use of protective crops to shield asparagus plants, chemical control of the flies early in the morning by means of a rapid contact poison (nicotine dust was found effective) and the prompt removal of infested stumps or stems.

VIII. SUMMARY

1. The history and identification of the asparagus miner, *Melanagromyza simplex* H. Loew, are outlined. The fly was described in 1869, and associated with asparagus in the United States of America in 1896 and 1897. In Europe it was recognized about the same date.

2. The salient points of the morphology of all stages are briefly described.

3. Its geographical range is the United States of America and Europe. In Europe it has been reported from Hungary, France, Germany and England. In U.S.A. the most numerous records are from the eastern states but it has also been reported from Iowa and California.

4. There are two generations a year at Harpenden, England. The flies of the first flight are on the wing from early June to the end of July, those of the second from the beginning of August until about the second week in September. Details of the life history are given.

5. The damage caused by the larvae mining the stems of asparagus is not considered important in cutting beds, although seedling beds might suffer more. When, however, the mining is followed by an attack by the larvae of *Lonchaea flavidipennis* Zett. the damage is more important.

6. An attempt to assess the local abundance of the flies by direct field observation is described.

7. Three parasites—the braconid, *Dacnusa ?bathyzona* Marsh.; a pteromalid, *Sphegigaster* sp.; and a eulophid, *Pleurotropis epigonus* Walk.—have been identified by Dr Ferrière and in addition an unidentified parasite has been reared. Brief observational notes have been given concerning these parasites. It is noted that *Dacnusa rondanii* Giard has previously been recorded as a parasite.

8. Control measures recommended by other workers have been mentioned.

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EXPLANATION OF PLATES XXXVI AND XXXVII

Illustrating the biology of *Melanagromyza simplex* H. Loew

PLATE XXXVI

- Fig. 1. Two eggs *in situ*. × 60.
- Fig. 2. 1st instar larva. × 60.
- Fig. 3. 2nd instar larva. × 33.
- Fig. 4. 3rd instar larva. × 33.

PLATE XXXVII

- Fig. 1. Over-wintering puparia at base of stem. Nat. size.
- Fig. 2. Mine with puparium in young growth. Nat. size.
- Fig. 3. Puparia on the stem in summer. Nat. size.
- Fig. 4. Adult female fly with outstretched wings. × 10.
- Fig. 5. Adult female fly with wings in resting position, the abdomen has shrunk. × 10.
- Fig. 6. Living female fly. Nat. size.

(Received 28 November 1936)



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 1.



Fig. 2



Fig. 3



Fig. 4.



Fig. 4.



Fig. 5.

THE HOLLYHOCK SEED MOTH (*PLATYEDRA MALVELLA* HÜBN.), TOGETHER WITH NOTES ON THE DISTRIBUTION OF *APION RADIOLUS* KIRBY AND AN ASSOCIATED *CLINODIPLOSIS* SPECIES

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(With Plate XXXVIII and 1 Text-figure)

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I. INTRODUCTION

IN the late summer of 1934 it was noticed that hollyhock seeds in Harpenden were suffering greatly from attacks by a lepidopterous larva which ate its way through the centre of the seeds and so passed almost round the entire seed-head. As the writer was interested in finding suitable insects for studying the fluctuations in insect numbers from year to year, it was decided to investigate this insect as a possible subject for such a study.

The following paper deals with the bionomics and distribution of the insect, *Platyedra malvella* Hübn., and includes notes on the distribution of two other insects found in hollyhock seed-heads, viz. *Apion radiolus* Kirby (a weevil) and a *Clinodiplosis* species (a gall midge).

II. IDENTIFICATION AND HISTORY

The lepidopterous larva was recognized as being closely related to *Platyedra gossypiella*, and when moths were reared they were sent to Mr E. Meyrick who kindly confirmed their suspected identity as *Platyedra malvella* Hübner. Mr Meyrick has written to the writer informing him that the genus *Platyedra*, which contains all those species feeding on seeds of malvaceous plants, is characterized by the presence of a pecten of scales on the scape. There are about half a dozen species in this genus centred around the Mediterranean. The most notorious species is *P. gossypiella*, the pink boll-worm of cotton.

Little has been written about this insect. *P. malvella* was originally described by Hübner as *Gelechia malvella*. Meyrick⁽⁴⁾ described it as follows: "17-19 mm. Head and palpi whitish ochreous. Forewings pale brownish, becoming fuscous posteriorly, with traces of darker strigulae, more distinct and blackish on costa; stigmata rather dark fuscous, first discal beyond plical, second sometimes obsolete; a very indistinct suffused pale fascia at $\frac{3}{4}$. Hindwings light grey." The general appearance of the moth is shown in Pl. XXXVIII, fig. 1.

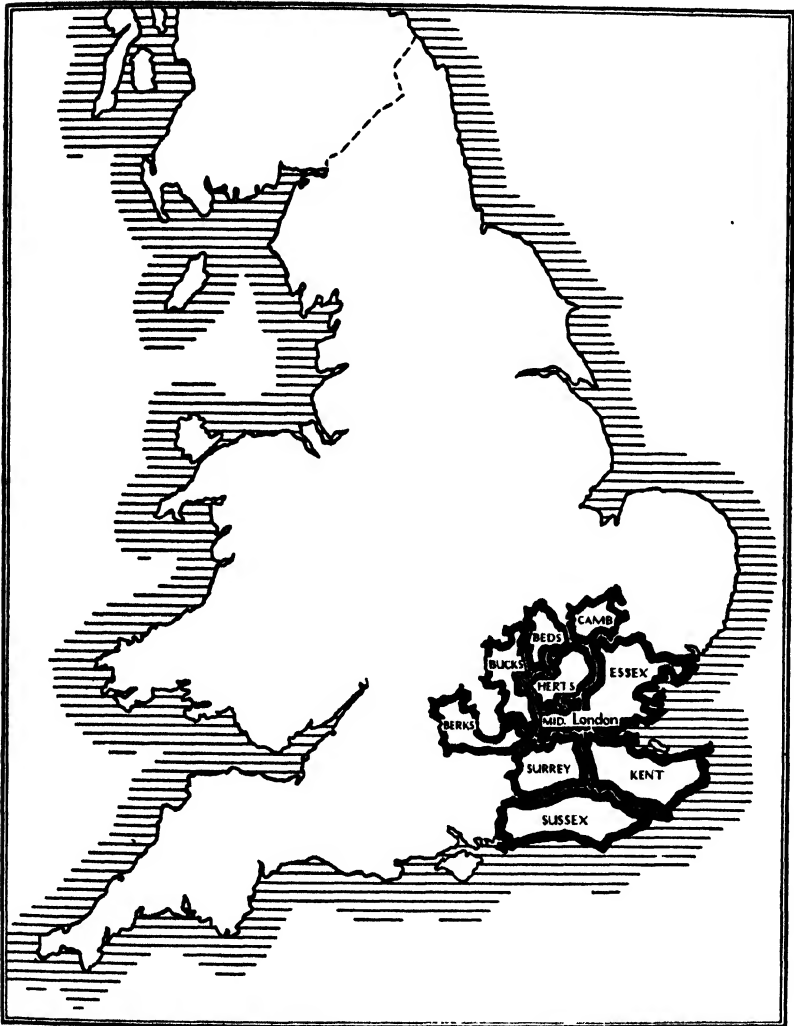
Amongst the economic references, Zacher⁽⁶⁾ dealt with it as a pest of medicinal plants in Germany. Belski⁽¹⁾ listed it on *Althaea officinalis* in a list of pests observed on medicinal plants in the neighbourhood of Kiev, and Gavalov⁽²⁾ mentioned it on cultivated *A. rosea* in the Crimea. Helm⁽³⁾ recorded it from Saxony as infesting the seed-capsules of hollyhock (*Althaea*) and Rekach⁽⁵⁾ recorded it in Transcaucasia infesting cotton and stated that it caused 16.7 per cent loss of crop. Experiments showed that 90-95 per cent control could be obtained by dusting with Paris green or sodium fluosilicate.

Apart from the use of hollyhock as a medicinal plant, the writer has been informed by one large seedsman that these plants are worth growing for the sale of the seed.

III. DISTRIBUTION

This insect has been recorded from central and southern Europe and Russia. Meyrick⁽⁴⁾ stated that in England it occurs locally as far north as York. Mr A. E. Wright (*in litt.*) has referred the writer to a record in Ellis's Lancashire and Cheshire List, copied from Stainton's Manual, of its occurrence at Manchester; in Porritt's Yorkshire List, of its occurrence at Huddersfield and Scarborough (copied from Stainton's Manual); and in Hayward's Derbyshire List, a doubtful record from Burton. These

records are old and require confirmation. There is also a record (55th, 56th and 57th *Ann. Rep. Lancs. Chesh. ent. Soc.* p. 203) of its capture at Grange-over-Sands, Lancs by A. E. Wright.



Text-fig. 1. Distribution of *Platyedra malvella* Hüb. in 1934 and 1935.

A detailed survey of the distribution of *Platyedra malvella* in Great Britain was attempted. Many persons, indicated by their initials in Table I, took part in this survey and sent the writer samples of hollyhock seed-heads. He takes this opportunity of reiterating his thanks to

them for their interest and help without which it would have been impossible to carry out the work. Requests for samples were sent out in 1934 and 1935. Heavy infestations of the larvae were apparent, each year, in Bedfordshire, Berkshire, Buckinghamshire, Essex, Cambridgeshire, Hertfordshire, Kent, Middlesex, Surrey and Sussex. Not a single specimen was found in any sample either year from any other county. A few samples were received from Wales, Ireland and Scotland, but here again the larvae were not present nor were there any traces of their damage. It would seem therefore that nowadays at any rate the moth is restricted in its breeding to the south-eastern counties of England (Text-fig. 1).

Table I

Survey of distribution of *Platyedra malvella* *Hüb.*, *Apion radiolus* *and* *Clinodiplosis* *sp.* *on hollyhocks in England, 1934 and 1935*

County	Locality, date and collector's initia	<i>P. malvella</i>	<i>A. radiolus</i>	<i>Clinodiplosis</i> <i>sp.</i>
Bedfordshire	Bedford:			
	5. x. 34, C.W.H.	x	.	x
	viii. 35, C.W.H.	x	x	x
Berkshire	Reading:			
	18. ix. 34, M.A.F.S.	x	x	.
	viii. 35, M.A.F.S.	x	.	x
Buckinghamshire	High Wycombe:			
	20. ix. 34, L.G.B.	x	.	.
	27. viii. 35, L.G.B.	x	.	.
Cambridgeshire	Cambridge:			
	11. ix. 34, I.T.	x	x	.
	viii. 35, I.T.	x	x	.
Cheshire	Birkenhead:			
	29. ix. 34, A.W.	.	.	.
	13. x. 34, A.W.	.	.	.
	Bramhall			
	24. ix. 35, C.H.-S.	.	x	.
	Penyffordd, near Chester:			
	1. ix. 35, W.M.D.	.	.	x
Cornwall	Launceston:			
	15. x. 34, E.G.T.R.	.	.	.
Cumberland	Edenhall:			
	29. viii. 35, H.B.	.	.	x
Derbyshire	Ilkeston:			
	6. x. 35, S.E.M.	.	.	x
	Langworth:			
	6. x. 35, S.E.M.	.	.	.
Devon	Barnstaple:			
	22. ix. 34, E.G.H.	.	.	.
	21. viii. 35, E.G.H.	.	.	x
	Newton Abbott:			
	13. ix. 34, L.N.S.	.	.	.
Dorset	Bridport:			
	21. ix. 34, S.E.B.	.	.	.
	Chickerell, Weymouth:			
	15. ix. 34, C.S.F.	.	x	.
	26. viii. 35, C.S.F.	.	.	.

Table I (cont.)

County	Locality, date and collector's initials	<i>P. malvella</i>	<i>A. radiolus</i>	<i>Clino- diplosis</i> sp.
Durham	Newcastle: 31. x. 36, R.A.H.G.	.	.	×
Ely, Isle of	—	—	—	—
Essex	Stanford-le-Hope: 16. ix. 34, C.R.N.B. 28. viii. 35, C.R.N.B.	×	×	. ×
Gloucestershire	Bristol: 6. ix. 34, C.L.W. 18. ix. 34, A.W. 25. ix. 35, C.L.W. Longford: 28. ix. 34, C.L.W.
Hampshire	Bitterne Park, Southampton: 8. x. 34, C.G.J. Highfield, Southampton: 21. ix. 34, C.G.J. Shirley, Southampton: 21. ix. 34, C.G.J. Westend, Southampton: 8. x. 34, C.G.J. ×	×
Herefordshire	Hereford: 21. ix. 35, C.L.W.	.	.	.
Hertfordshire	Harpenden: (1) 20. viii. 34, H.F.B. (2) 26. viii. 34, H.F.B. (3) 29. viii. 34, C.B.W. (4) 13. ix. 34, V.S. (5) 2-19. viii. 35, H.F.B. (6) 2. viii. 35, A.C.E. (7) 10. viii. 35, C.B.W. (8) 15. viii. 35, H.F.B. (9) 16. viii. 35, M.D. Letchworth: 15. ix. 34, F.W.E. viii. 35, F.W.E. Radlett: 2. ix. 35, A.M.C. St Albans: 9. viii. 35, A.D. Welwyn G. C.: 28. viii. 34, S.E.B.	× × ×
Huntingdonshire	—	—	—	—
Kent	Hythe: 15. x. 34, G.K.D. Wye: 17. ix. 34, S.G.J. 29. ix. 35, R.T.P.	×	. ×	×
Lancashire	Burnley: 17. x. 34, W.G.C. Failsworth, Manchester: 28. ix. 34, F.P. Mosely: 9. ix. 35, H.B.
Leicestershire	Kegworth: 30. viii. 35, A.R. Loughborough: 6. x. 35, S.E.M. ×	. . . ×

Table I (cont.)

County	Locality, date and collector's initials	<i>P. malvella</i>	<i>A. radiolus</i>	<i>Clino-diplosis</i> sp.
Lincolnshire	Caistor:			
	1. ix. 35, A.R.	.	.	.
London	West Norwood:			
	2. ix. 35, H.E.T.	x	.	x
	Tulse Hill:			
	8. x. 35, R.M.G.	x	x	x
Middlesex	Uxbridge:			
	31. viii. 34, D.C.T.	x	x	.
	30. ix. 35, D.C.T.	x	x	x
Monmouthshire	—	—	—	—
Norfolk	Attleborough:			
	16. x. 34, J.C.	.	.	.
	Downham:			
	27. ix. 34, E.R.P.	.	.	.
	Norwich:			
	9. ix. 35, C.B.W.	.	.	x
	Saham Toney, Thetford:			
	ix. 35, M.G.N.	.	.	.
Northamptonshire	Cottesbrook Hall:			
	2. xi. 34, E.T.W.S.	.	.	x
Northumberland	Newcastle:			
	25. x. 34, R.A.H.G.	.	.	.
Nottinghamshire	Nottingham:			
	6. x. 35, S.E.M.	.	.	.
	Sutton Bonington:			
	11. ix. 34, A.R.	.	x	.
	30. viii. 35, A.R.	.	.	.
Oxfordshire	Oxford:			
	17. ix. 34, M.R.	.	x	.
	9. x. 34, G.D.H.C.	.	.	.
	23. viii. 35, J.J.W.	.	.	x
	viii. 35, M.R.	.	.	x
	22. viii. 35, G.D.H.C.	.	.	x
Rutland	—	—	—	—
Shropshire	Wellington:			
	30. viii. 35, H.C.F.N.	.	.	x
Somerset	Bath:			
	14. x. 34, C.L.W.	.	.	.
	Dundry:			
	2. x. 34, L.O.	.	x	x
	Williton:			
	15. ix. 34, J.R.	.	.	.
	viii. 35, J.R.	.	x	x
	23. ix. 35, J.R.	.	.	.
Staffordshire	—	—	—	—
Suffolk	Bury St Edmunds:			
	viii. 35, R.S.W.	.	x	x
Surrey	Byfleet:			
	17. ix. 34, G.F.W.	x	.	.
	17. viii. 35, G.F.W.	x	.	x
	West Byfleet:			
	17. ix. 34, G.F.W.	x	.	.
	17. viii. 35, G.F.W.	x	x	.

Table I (cont.)

County	Locality, date and collector's initials	<i>P. malvella</i>	<i>A. radiolus</i>	<i>Clinodiplosis</i> sp.
Sussex	Rottingdean:			
	25. ix. 34, R.N.C.W.	x	.	.
	ix. 35, R.N.C.W.	x	.	x
	Worthing:			
	28. ix. 34, F.C.W.	x	.	.
	1. x. 34, F.C.W.	x	.	x
	21. viii. 35, F.C.W.	x	.	x
Warwickshire	—	—	—	—
Westmorland	—	—	—	—
Wight, Isle of	—	—	—	—
Wiltshire	Marlborough:			
	24. x. 34, G.P.	.	.	x
	1. x. 35, G.P.	.	.	x
Worcestershire	Henwick:			
	28. ix. 34, W.D.S.	.	.	.
	Worcester:			
	ix. 35, W.D.S.	.	.	.
Yorkshire	Bingley:			
	2. ix. 35, R.B.D.	.	.	x
	Huddersfield:			
	13. ix. 34, M.E.M.	.	.	.
	Leeds:			
	18. ix. 34, C.W.G.	.	.	.
	ix. 35, C.W.G.	.	.	x

About 6000 hollyhock seed-heads were examined in 1934 and about 4000 in 1935. An *Apion* weevil and a gall midge larva, *Clinodiplosis* sp., were commonly present. The weevil has since been kindly identified for the writer as *Apion radiolus* Kirby by Dr K. G. Blair.

In Table I the distribution of *Platyedra malvella*, *Apion radiolus*, and the *Clinodiplosis* species is shown. It can be readily seen that whereas *Platyedra malvella* is definitely restricted in its distribution, *Apion radiolus* and the *Clinodiplosis* species on the other hand occur generally throughout England. The *Clinodiplosis* also occurs in Wales and Ireland.

IV. BIONOMICS

(a) Damage

The damage caused by the larvae of *Platyedra malvella* is the destruction of the seed (Pl. XXXVIII, fig. 2). The larvae eat right through the centre of each seed and sometimes progress right round all the seeds in a single seed-head (i.e. all the seeds resulting from one flower), thus enabling the seed remnants to be threaded as shown at the top of fig. 2. The holes in the seeds are characteristic, smaller holes by the younger larvae, large ones by the nearly full-grown larvae. The damage by the *Apion* is different, the edges of the seed being eaten in irregular pieces.

(b) Host plants

Platyedra malvella most commonly attacks the seed of hollyhock (*Althaea rosea*), though it is recorded also as feeding on the seeds of common marsh mallow (*A. officinalis*). The writer has found one record of it attacking cotton buds, ovaries and bolls (Rekach (5)).

(c) Life cycle

The moths are on the wing at Harpenden, Herts, from the third week in June until the beginning of August. They seem to emerge at all hours of the day from 7 a.m. until 8 p.m. (Greenwich Sun Time). The moths live for at least a week, probably considerably longer, specimens kept in glass tubes plugged with cotton-wool having lived for from 3 to 9 days. One individual died after 3 days, two after 4 days, two after 5 days, six after 6 days, one after 7 days, one after 8 days and two after 9 days.

The moths have been observed ovipositing on the sepals both before the flowers have opened and also after the petals have started to shrivel.

First instar larvae (just over 1 mm. in length) have been found at the base of the petals, among the stamens, on the surface of the young seed, on the sepals and in one case inside the staminal tube.

The newly hatched larvae feed on the top of the seeds forming slight channels, but very quickly burrow to the centre of a seed and then pass from one seed to another, eating out the centre of each, until they have perforated almost every seed in the seed-head. By this time they are full grown. Sometimes two or more larvae are found in a single seed-head.

The larvae grow in size quickly and by the third week in August are full grown, measuring about 1 cm. long. They are whitish ochreous with red spots, the head being blackish. The scutellum on the prothorax is obvious and dark. Larvae of all sizes can be found in the seed-heads in the middle of August as the period of emergence of the moths and consequently of oviposition is prolonged.

When fully fed the larvae descend to the soil where they pass the winter in over-wintering cocoons. Occasionally the larvae eat an exit hole through one of the sepals (Pl. XXXVIII, fig. 2), at other times no definite hole is made. On a few occasions these cocoons have been found actually in the seed-heads between the seeds and the sepals, but this is not the usual position. Rolls of corrugated cardboard have been used successfully to obtain cocoons free from soil, the larvae spinning up in them readily.

After the winter the larvae again become active, the habits thus closely resembling those of *Platyedra gossypiella*, and tunnel about in the soil. The illustration (Pl. XXXVIII, fig. 3), shows the larvae in their tunnels in the soil. In the outdoor insectary at Harpenden this post-winter activity took place in 1935 and 1936 throughout May. It was unexpected to find that, although the larvae in the soil might have been expected to be negatively phototropic, many of them came to the sides of the lamp-glasses in which they were kept and so were clearly visible. This movement to the sides might, however, be due not so much to the attraction of light as to the pressure obtained by close proximity to the glass.

Table II
Comparison of degree of infestation 1934 and 1935

County	Locality	No. and size of sample, i.e. no. of seed-heads	1934 % attack	1935 % attack	No. and size of sample, i.e. no. of seed-heads
Bedfordshire	Bedford	(i) 50	26	69	26 (ii)
				48	100 (iii)
				56	75 (iv)
				83	75 (iv)
				53	100 (vi)
				68	100 (vii)
Berkshire	Reading	(i) 142	58	42	200 (n)
				53	125 (m)
Buckinghamshire	High Wycombe	(i) 20	25	6	47 (ii)
Cambridgeshire	Cambridge	(i) 175	95	90	102 (i)
				47	93 (n)
Essex	Stanford-le-Hope	(i) 24	100	94	130 (ii)
Hertfordshire	Harpenden	(i) 200	88	60	58 (iv a)
		(ii) 76	93	70	50 (iv b)
		(iii) 518	93	78	50 (iv c)
				82	50 (iv d)
				94	250 (v)
				94	100 (vi)
				84	50 (vii)
				74	50 (viii)
	Letchworth	(i) 120	25	70	100 (ii)
	Radlett	—	—	46	200 (i)
Kent	St Albans	—	—	99	100 (i)
	Wye	(i) 94	0	60	200 (m)
		(ii) 62	0	—	—
	Hythe	(i) 127	39	—	—
London	West Norwood	—	—	83	161 (i)
	Tulse Hill	—	—	78	100 (n)
Middlesex	Uxbridge	(i) 873	97	88	200 (i)
Surrey	Byfleet	(i) 101	6	18	76 (i)
	West Byfleet	(i) 21	57	44	64 (i)
Sussex	Brighton	(i) 28	96	91	75 (i)
	Worthing	(i) 60	82	46	90 (iii)
		(ii) 36	58	—	—

Towards the end of May and throughout June the larvae spin the cocoons in which they pupate. The pupae are pale brown and about 0.75 cm. long. The moths emerge from these pupae at the end of June until the beginning of August. There is only one generation a year.

(d) *Degree of infestation*

In 1934 and 1935 samples of seed-heads were obtained from many places. In Table II the comparative infestations are set out. It will be seen that even when large numbers of seed-heads have been examined, very heavy infestations have sometimes been found, e.g. out of 873 heads obtained from Uxbridge in 1934 97 per cent showed signs of damage. Averaging all the samples examined in 1934 and 1935 separately for each year, the degree of infestation works out at 65 per cent in each instance.

V. PARASITE

Only one species of parasite has been reared from the larvae of this moth. It has been kindly identified for the writer by Dr C. Ferrière as *Angitia rufipes* Grav. (Ichneumonidae). A male and female are illustrated in Pl. XXXVIII, fig. 4.

The numbers reared have been small, five only being bred in 1935 and six in 1936, although several scores of moths were reared each year. The parasite occurred in an Essex sample and in several from Harpenden.

VI. SUMMARY

A survey has been made of the distribution of *Platyedra malvella* Hübn. in Great Britain and it has been found that the larvae are restricted on hollyhock to the south-eastern counties of England.

Apion radiolus Kirby and a gall midge *Clinodiplosis* species which are also found in the seed-heads of hollyhock are generally distributed throughout England; the *Clinodiplosis* species also occurs in Wales and Ireland.

The life cycle of *Platyedra malvella* has been described. There is only one generation a year. The moths are on the wing late in June until the beginning of August. The larvae feed on the seeds of hollyhock perforating the seeds characteristically. When full grown from the middle of August onwards, the larvae descend to the soil where they spend the winter in cocoons. In May the larvae become active again and tunnel through the soil. They spin up again towards the end of May and in June for pupation. The host plants are *Althaea rosea*, *A. officinalis* and there is at least one record on cotton.



Fig. 1.



Fig. 2.



Fig. 3.

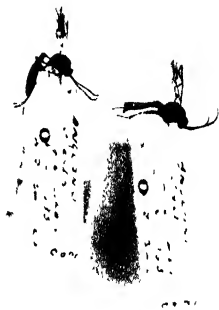


Fig. 4.

The degree of infestation of hollyhock seed-heads in 1934 and 1935 is shown in a table.

An Ichneumonid parasite, *Angitia rufipes* Grav., has been reared in small numbers from Essex and Hertfordshire material.

The literature concerning the economic importance of this moth has been reviewed.

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EXPLANATION OF PLATE XXXVIII

Fig. 1. Adults of *Platyedra malvella* Hübn. Nat. size.

Fig. 2. Damage to hollyhock seeds by larvae. Slightly reduced.

Fig. 3. Post-winter activity of larvae in soil. Nat. size.

Fig. 4. Male (right) and female (left) *Angitia rufipes* Grav., the parasite of *P. malvella* Hübn.
× nearly 2.

(Received 5 December 1936)

AN HISTORICAL STUDY OF THE MIGRATIONS OF *CELERIO LINEATA LINEATA* FAB. AND *CELERIO LINEATA LIVORNICA* ESP. (LEPIDOPTERA)

By Mrs. K. J. GRANT.

(*Rothamsted Experimental Station.*)

[Read 5th May, 1937.]

SINCE early in 1931 the South-Eastern Union of Scientific Societies has been encouraging the collection of records dealing with the migration of insects in the British Isles, and a special Insect Immigration Committee has been set up to organise the efforts of amateurs and others who are interested in this branch of entomology. As a result of the Committee's activity, a great deal of useful information is collected yearly and sent to Rothamsted Experimental Station, where it is available for examination and analysis. Some very interesting facts and theories are already beginning to emerge from this steadily accumulating mass of data, but it suffers somewhat from the drawback of dealing only with current events, and many years must pass before one can expect by its means to throw much light on underlying problems such as periodicity, correlation with climatic factors, or the relationship between migrations in the British Isles and those in other parts of the world.

Another store of information is, however, available in all those periodicals and journals which have for a century or more been in the habit of devoting part of their space to records of the observations of collectors and naturalists in all parts of the world. From these it seemed that it might be worth while to make an investigation into the past history of one or two individual species for comparison with the more recent records of the Insect Immigration Committee.

In 1931 *C. lineata* var. *livornica* became unusually common in western Europe, and was reported to the Insect Immigration Committee in greater numbers than it had ever before been recorded in Great Britain. This led to its selection for the present investigation, and it has proved to be a very suitable subject in many ways. Not only is it rare enough in this country to have received a good deal of attention from collectors, but in other parts of the world its larvae are occasionally a pest of vines and other cultivated plants, and its years of unusual abundance have therefore been recorded from time to time in the economic literature. In addition, the information available is sufficiently abundant to provide an adequate basis for investigation, without being so copious as to prove unmanageable. In fact the species might almost have been designed to suit the purposes of the work in hand.

Distribution.

The moth generally known in this country as *Celerio* (*Deilephila*, *Phryxus*) *livornica* Esp. is the old-world sub-species of the Sphingid *Celerio lineata* Fab., the nominal form being confined to America. A third sub-species, *Celerio l. livornicoides* Lucas, is found in Australia, but so little is known about it, and it has so seldom been recorded in any abundance, that I do not feel justified in dealing with it here.

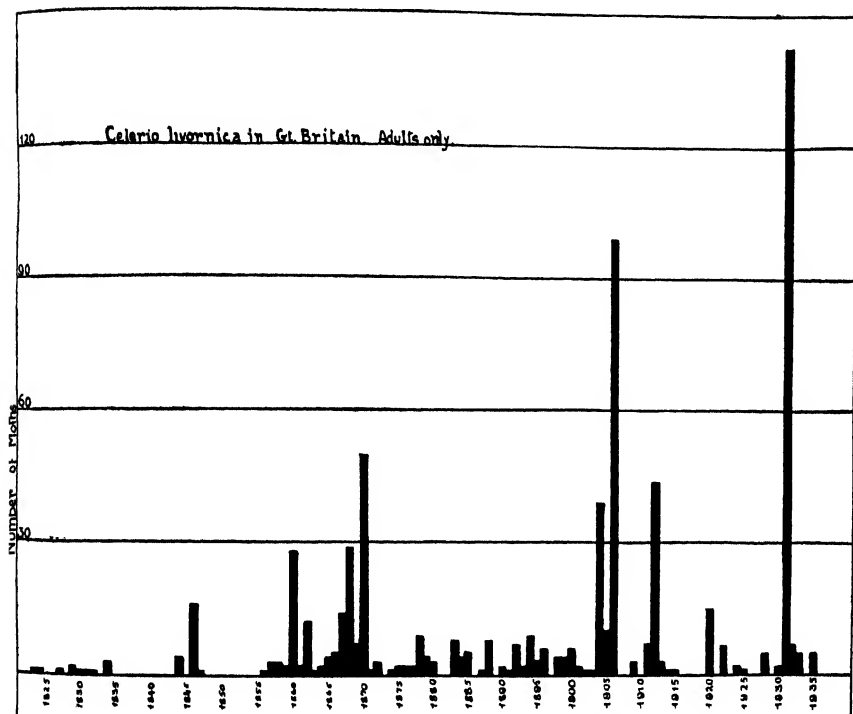
C. lineata and *C. l. livornica* are very similar both in appearance and habit, but they appear to form perfectly distinct geographical races, and there is sufficient difference in their markings and gross morphological detail to prevent their confusion when seen side by side. *C. lineata* has been seen from New Jersey to Ecuador, and *C. l. livornica* from China to western Europe and southward to the Orange Free State, yet in neither case is the permanent habitat (if any) known with any degree of certainty. In some of the warm-temperate parts of the northern hemisphere they have been seen frequently enough to give the casual observer the impression that they are indigenous, but a closer examination of the literature suggests that they are usually migrants from elsewhere. *C. l. livornica*, for example, is fairly frequently seen in the African littoral of the Mediterranean, and occasionally appears there in great abundance as a pest. Many years may pass, however, without a single record coming from this area, and when the larvae ravage the vineyards in years of abundance, the cultivators seem to regard them as an unusual pest. In various parts of Europe the moths have been abundant in about one-third of the past sixty years, and small numbers have occurred more often, yet the species has nowhere permanently established itself, and it may be absent for years on end. The same seems to be true of the American sub-species, which is common in some seasons in many parts of the United States, but can hardly be said with certainty to be indigenous in any particular district.

Outbreaks of both sub-species are most frequent and abundant in the neighbourhood of deserts and semi-desert areas; for example, California, Utah, Arizona and other States surrounding the Great American Desert; Morocco, Algiers and Tunis, near the Sahara; and Anatolia, the Caucasus, southern Russia, and Persia, near the deserts and arid steppes of Asia Minor. The only reference I have been able to find to an outbreak in South America took place in 1925 in the Santa Elena Peninsula, a desert district in Ecuador, during the first rains that had fallen for several years. One may perhaps venture to suggest that one is dealing with a desert species which, in response to unusual conditions of climate or to some similar stimulus, sometimes outbreeds the resources of its normal habitat, and is forced to migrate elsewhere. American entomologists would probably agree with this view, since they often find *lineata* especially common on the outskirts of deserts in its years of abnormal abundance. The habits of the larvae are quite compatible with this supposition. They occur on all sorts of low-growing plants and weeds, and not only the species but even individuals are extremely polyphagous. On several occasions it has been observed that when a large number of larvae have completely destroyed the food plants on which they were hatched, they will migrate gregariously, examining everything in their path, until they have found a sufficient growth of something suited to their taste; this may be a species belonging to a different family or even to a different natural order from that on which they started life. Behaviour such as this would have a definite survival value for a species dependent for its food supply upon the sparse flora of desert areas. An interesting account of such a mass migration of larvae is given by J. Brunetau (1932, *Rev. Zool. agric.* 31 : 9), and may be translated as follows: "In the commune of Bors de Baignes quantities of larvae devastated about a hectare of buckwheat, leaving only the stalks and reducing the plantation to skeletons. Lacking food the ravagers descended to the ground in millions, and moved off browsing on *Rumex* by the way, until they reached a plantation of cabbages. Some of them climbed on to these plants, but they were soon abandoned, and at last they reached a vineyard, which they began to misuse. Parts recently sulphated

were respected, but the young growth, the leaves where the Bordeaux mixture was less apparent and even the twigs were damaged."

Celerio livornica in Britain.

Whatever may be the stimulus which releases the migratory impulse, it is very strong when once aroused. If Africa be the source of the majority of specimens of *livornica* caught in Great Britain in spring, a fact which there is little reason to doubt, journeys of a thousand miles or more must frequently have



1

been flown. Great Britain has been reached in at least seventy-two of the 113 years since the species was first found here (fig. 1), and although in most of these years only one or two individuals have been seen, there have been occasions when comparatively large numbers must have been present. In 1870, for example, at least seventy moths and larvae were recorded; in 1906 the numbers reached one hundred and in 1931 over one hundred and fifty individuals were seen, among them both adults and larvae. It should not be thought that these rising figures represent an actual increase in the numbers reaching Britain; they are much more likely to indicate the larger number of persons taking an interest in the matter. But it is reasonable to suppose that fig. 1 does give a more or less true picture of the relative abundance and scarcity in various years. It is unlikely, for example, that the number of observers decreased materially

Celerio Livornica in Britain—(Larvae in Brackels)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	No. Date	Years Total
1825						1								1
24						1								1
27													1	1
29													2	2
1830													1	1
31							1							1
32							1							1
34							3*(2)							3*(2)
1844						1								1
46				9	1	2							4	16
47													1	1
1856													1	1
57													3	3
58				1	1	1							3	3
59													2	2
1860					18	1	6						3	28
61	1	1												2
62			1	3	6	1							1	12
63						1							2	1
64													2	2
65								2	1				1	4
66					2								3*(4)	5*(4)
67					8		1						5	14
68				1			2	20	2				4	29
69					3			1					5	7
1870				1	11	12	1*(10)	11	7	1			6*(10)	30*(20)
71													1	1
72						1			1				1	3
74						1							1	1
75				1									1	2
76													2	2
77						1		1					2	2
78						5	1	3					8	9
79						4							4	4
1880									2				1	3
83						2	4						2	8
84						1	2		1				5	4
85													5	5
87			1											1
88						6							2	8
1890								2					2	2
91									1					1
92					1	5							1	7
93					2									2
94						8	1							2
95						1	1		1					3
96					3		1	1					1	6
98					1	1				1			1	4
99					3			1						4
1900					2	1	2						1	6
1						1		1						2
2							(1)						1	1
3			1											1*(1)
4					3	1	(1)	1	4					1
5				7	3	1							2	39*(1)
6					19	69	1*(1)	2	6				2	10
9				1			1			1			2	99*(1)
11						1	1	2						3
12	1	1			4								1	7
13						2			1				2	44
15					1									3
16					1									1
1920					14	(4)	1							1
22							(1)						1*(1)	6*(1)
24					1		1							2
25					1			(1)						1*(1)
28				1		1	1		2					5
1930								2						2
31					31	100	1*(66)	(12)	1				9	142*(23)
32						2	(5)						5	7*(5)
33							(6)	2	1		2			5*(6)
35				1	1			1					2	7
Adults	2	2	2	25	209	240	34	50	36	4	2	0	87	693
Larvae						1	43	15					12	69

from 1906 to 1907, yet the number of *livornica* recorded fell from one hundred to nil; again, from 1931 to 1934 the number of observers reporting the presence of migrants to the Insect Immigration Committee rose steadily, yet records of *livornica* fell from more than 150 in 1931 to 12 in 1932, 11 in 1933, and none in 1934.

Fig. 2 shows the seasonal distribution of all the individuals shown in fig. 1, with the addition of larvae, the numbers of which are given in brackets. In some cases, the date of capture, apart from the year, is not known; such insects are shown in the column before the year's total.

It will be seen that the species has been found in every month of the year except December; May and June being those in which it is most numerous. Larvae are more common than adults in July, and the subsequent increase in the number of adults in August and September may be taken as evidence of the emergence of a British-born generation. An interesting comparison may be made between *livornica* and those migrants which, like *Vanessa atalanta*, *Vanessa cardui*, *Plusia gamma*, etc., visit this country every year in fairly large numbers. In these cases the autumn emergences are almost invariably far more numerous than the spring arrivals, showing that our summer conditions are suitable, and that it is only inability to pass the winter here that prevents the species from gaining a permanent foothold. In *livornica*, on the other hand, the autumn generation is rarer than that of early summer; our conditions must be unfavourable to the species even at the best time of year, and even should they succeed in passing a winter or two, they must die out in time unless reinforced by fresh migrants from the south.

The number of moths found in Great Britain from November to March is over 1% of the total, a surprisingly large amount. If *V. atalanta* were found in similar proportions, for example, many hundreds would be seen here every winter. While most of those found in April may be early immigrants (the species is active in Mediterranean districts in March) the occurrence of even single specimens in January and February suggests that hibernation is not entirely impossible, even if it be infrequent. The experience of those who have reared the species in captivity shows that the pupal stage is short, the moths emerging three or four weeks after pupation. There is no account of the finding of the pupa in winter in this country, or, indeed, anywhere else in Europe.

The occurrence of these unusually early individuals does not necessarily either precede or follow years of abundance; indeed, in one case a February specimen was caught during a period when the species was almost absent from Europe. This moth was shown at a meeting of the South London Entomological Society in 1887, and there can be no question of its correct identification. The account of its capture given by Mr. J. A. Helps (1887, *Entomologist*, 20 : 157) is so interesting that it is worth quoting here in full.

"On April 18th last an old servant, living with her father at Coles Cross, near Crewkerne, sent me a specimen of *Deilephila livornica*, which flew into their cottage (attracted by the light) in the beginning of February last. She states that they caught one last year, and that a neighbour took one the previous year, about the same date."

Apart from this one specimen (the other two mentioned in Mr. Helps' letter were not included in figs. 1 & 2, owing to lack of reliable identification) I have been able to trace no other record of *livornica* in 1887 in the whole of Europe, and only one in 1886, a single individual in Hesse, Germany.

The sex is recorded in eighty-five of the *C. l. livornica* found in Great Britain,

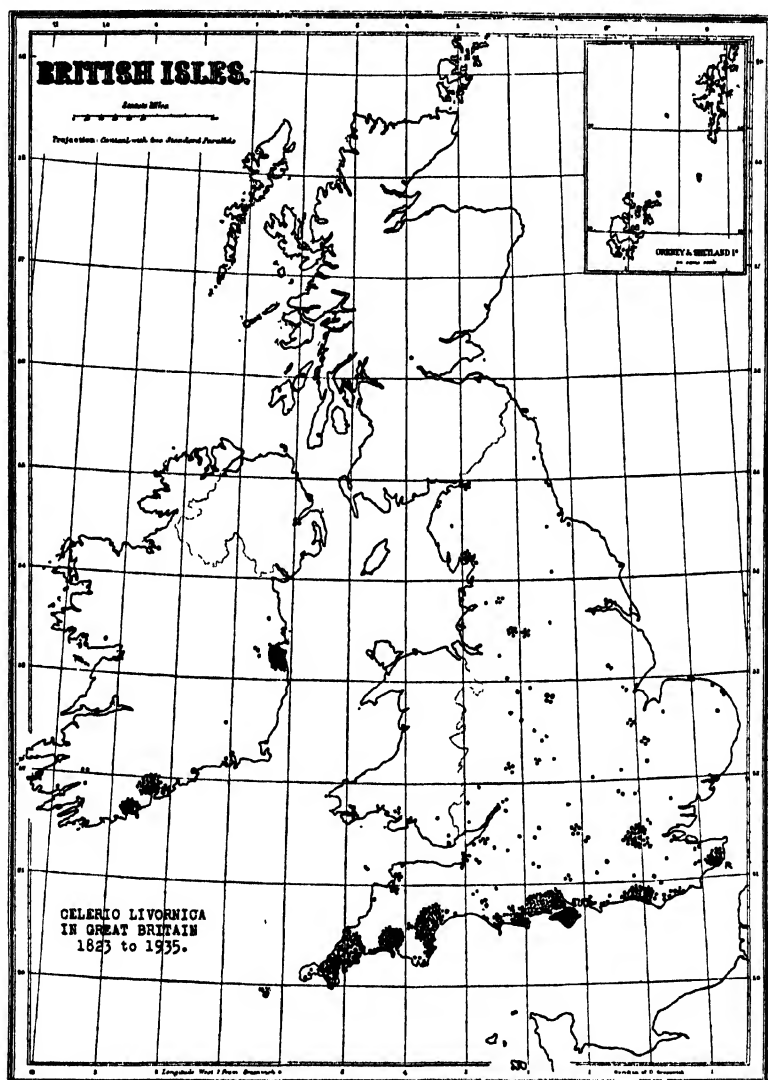
the proportions being fairly near to equality, *i.e.* forty males to forty-five females. Comparatively few of the continental specimens have been sexed, and in these the females outnumber the males by thirty-three to six. Part at least of the preponderance of females may be accounted for on the assumption that many individuals were only sexed at all as a result of their laying eggs, the sex in other cases being ignored. Nevertheless, there is some reason to suppose that the species is one in which the females normally outnumber the males. On one or two occasions, small numbers of imagines have been reared from eggs laid by captive females, and the sex of all the resulting moths has been recorded. In one such experiment, thirteen eggs gave rise to nine adults, eight of which were females, and in another an equal number of eggs gave rise to two males and four females. More information is needed on this point, and it is hoped that when next the species appears in quantity, observers will be careful to determine the sex in as many cases as possible.

The map in fig. 3 has been drawn to show the distribution of all *C. l. livornica* seen in Great Britain between 1823 and 1935, both adults and larvae, except for a few specimens whose locality I have been unable to trace. A separate map was originally drawn for the moths seen in late summer and autumn; it was thought that this might show a breeding area smaller than that reached by immigrants. This was not so, however; the two maps were essentially similar, and were eventually combined to form fig. 3, which now shows the location of all records, irrespective of season.

Naturally such a map is influenced by the distribution of human population. The large empty spaces shown in such areas as central Ireland and the mountains of Wales, where there are few people and fewer entomologists, probably received their quota of moths like other places, but the insects arrived unobserved and passed unseen. In estimating the local abundance of insects, however, there is one clue which does not depend on human population, and which may be used as a rough guide to relative numbers in different places. This is the number of insects seen by each individual observer. If this correction be applied to the present map, it is found that it only intensifies the tendencies that are already apparent. In the Midlands and North, the appearances are almost invariably solitary, and at intervals of many years. Along the south coast, the species occurs more frequently, and the same observer sometimes reports two or three individuals in a single year. But the greatest numbers are found in the extreme south-west and in Ireland. From Cornwall are records of as many as twenty-five specimens taken by a single collector in one year, while in Ireland, twenty-one *l. livornica* were seen at Timoleague, Co. Cork, in 1931, and thirty-two were reported from Shankhill, Co. Dublin, in the same year, by single observers in each case.

This tendency to greater abundance in the west is found in other migrants besides *l. livornica*, and gives rise to some interesting speculations. A glance at the map of Europe shows that if a migration leaves the western part of north Africa travelling northwards, after it passes Lat. 44° a part of it will have to cross the plains of France, and a part the Bay of Biscay. It is reasonable to suppose that the section flying over France will gradually thin out as it goes northwards, many individuals ceasing their migration and settling in France long before the Channel is reached. Those farther westward, however, will find themselves crossing the open sea, and will be unable to stop until they reach Cornwall, Ireland, or the shores of the Irish Channel. They will thus arrive with their numbers unimpaired, except by the loss of such individuals as have fallen into the sea and been drowned.

There is very little evidence of insects actually seen crossing the Bay of Biscay; one or two records do exist, however, and the arrival of butterflies in the Scilly Islands, where they are reported flying northwards in from the sea,



suggests that it is not impossible for them to do so. It may be objected that the four hundred miles from Spain is an unlikely distance for insects to fly at a single stretch, and that most individuals attempting to do so must inevitably perish, but it seems likely that migrating insects may be able to refresh themselves by floating for a while on the surface of the water. Miss

Longfield told me that she has seen a hawkmoth alight on the surface of a pool in Ireland, and rise from it again, and the keepers of lightships have on several occasions reported similar behaviour on the part of migrating Pierids. If this ability to rest on the water is at all normal, it must be of great assistance to Lepidoptera making long sea crossings.

C. l. livornica in Europe and *C. l. lineata* in America.

Celerio livornica was first described by Petiver from a specimen caught as long ago as 1698 in Lisbon (1764, *Gazophylacium* 1: 2, Tab. XL, Fig. 11).

The description is of the vaguest, but luckily the moth is figured well enough for identification. During the next century a few solitary adults of both subspecies were captured and described, and one or two larvae were found in vineyards. The first mention of large numbers occurs in 1818, when the species was plentiful in Switzerland especially round Berne (Meisner, 1818, *Nat. Anz.*, 1: 44). The moths were common in the Caucasus in 1829, and a single individual, probably on migration, was seen at a height of 12,000 feet on Mount

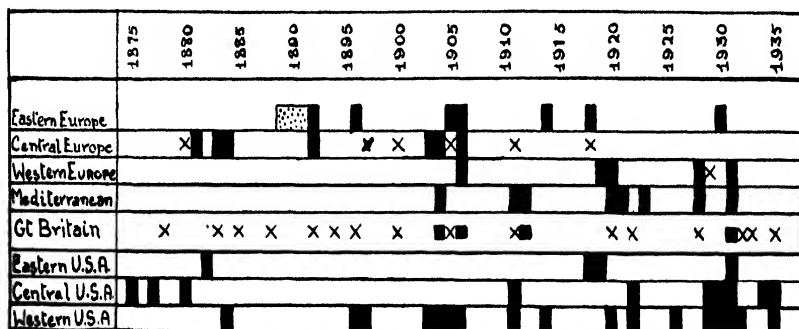


FIG. 4.—Comparison of outbreaks of *C. l. livornica* in Europe and *C. l. lineata* in America.

Elburz. In 1835 a migration arrived at Montpellier, on the south coast of France, after violent southerly winds; it is difficult to decide from the description whether the observer actually saw the moths coming in from the sea, but they were at any rate suddenly abundant all along the sea front.

In 1846 numbers appeared in Germany, and extended westwards as far as Great Britain, where twenty-six individuals were recorded. Twelve years later an outbreak in the Alburns (Elburz?) Mountains, near the Caspian, had no counterpart in western Europe, and in 1864 the moths were again common in Asia Minor, but not unusually so elsewhere. Western Europe was next visited in 1870, and on this occasion the greater number of records came from Great Britain; in 1874, the species was unusually abundant in Algiers, and present (numbers unspecified) in Persia. Outbreaks after this date are recorded in fig. 4, and need not be listed further in the text. That of 1883 is perhaps worth special reference, since in this year the moths were extraordinarily abundant all over central Europe, but apparently nowhere else.

It seems extraordinary that no mention of the damage done by the larvae should be made in European literature until 1904, although *C. lineata* had been recognised for some time in America as an occasionally severe pest of vines. The damage done, however, is very sporadic and local, and it was not until well into the nineteenth century that entomologists began to take an interest in the

practical aspects of their science; even then they largely confined themselves to the more important pests. It is probable that outbreaks occurred from time to time in Europe, but no one took any notice of them except the unfortunate farmers whose crops were attacked. Once attention was attracted to it, *l. livornica* was found to infest a great number of wild and domesticated plants. In cultivation, the most important host is the vine, and when the larvae are present in large numbers they will strip the plants very quickly, devouring even shoots and young grapes. Other plants besides vines are sometimes attacked; among them are olives (Tunis, 1904); cotton (Spain, 1928); buckwheat (Caucasus 1930 and France 1931). In addition to these hosts, the American sub-species has also been reported from tobacco (Jamaica, 1922).

Fig. 4 has been prepared to show in the form of a graph all outbreaks that have been traced in Europe and North America since 1875, the year when the first American outbreak was recorded. Particulars of most of these American outbreaks were sent me by the United States Department of Agriculture, but some half dozen additional records were obtained from various journals. The black areas in the graph represent years in which definite outbreaks occurred; the shaded area in eastern Europe for the years 1889-1891 represents a period when the species was certainly present in Rumania, but its numbers were recorded in such vague terms that it is impossible to say whether there was anything comparable with the outbreaks recorded in other places or not. The crosses represent the occurrence of the species in numbers which, although small, were unusually high for the latitude concerned; they may possibly have been connected with outbreaks farther south which passed unnoticed. The most surprising fact brought out by this figure is the degree of correspondence between years of outbreak in America and those in Europe; of the sixty years that have passed since the first outbreak was reported from America, there has been correspondence in forty years and dissimilarity in only twenty. The following table will make this clear:

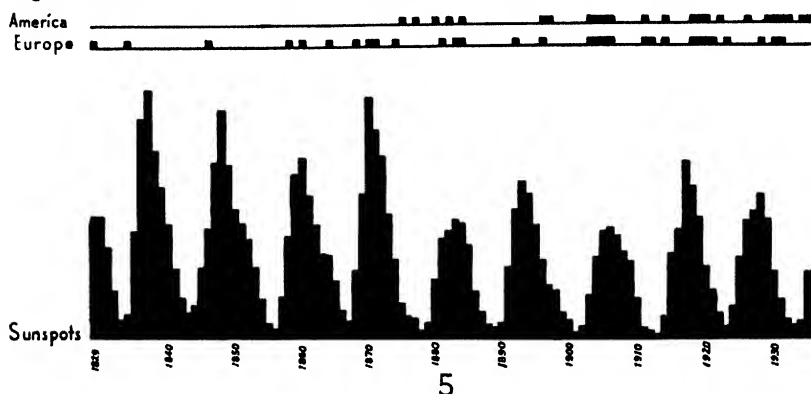
Outbreaks in both continents : 13	In America only : 10	In America : 23
In Europe only : 10	In neither : 27	Not in America : 37
In Europe : 23	Not in Europe : 37	Total : 60

Thus, of the twenty-three years when there were outbreaks in Europe, only ten years were without an outbreak in America; in the thirty-seven years when there were no outbreaks in Europe, they occurred only ten times in America. If the three doubtful years mentioned above, 1889, 1890, and 1891 be counted as years when no outbreak occurred, the correspondence becomes stronger, with forty-three years in which the continents resembled one another, and only seventeen in which they differed. It is most unlikely that figures such as these should be due to accident, the chances against it being about twenty-two to one if the three doubtful years in Rumania are counted as outbreaks, and greater than a hundred to one if they are not. It will be seen that the crosses representing small numbers in unlikely places tend to strengthen the correlation between Europe and America rather than to weaken it.

The implication of all this is clear. In seeking for an explanation of the

outbreaks of *I. livornica* and *I. lineata*, one must look for some large cause, wide enough in its effects to have influenced both continents simultaneously. One or two authors have, for example, put forward the suggestion that forest fires or the passing of locusts or grasshoppers may have caused the multiplication of the moths, by cutting back the larger trees and shrubs, and so encouraging the growth of the lower plants on which the larvae feed. But this will not satisfy the new requirements, since a forest fire in America will not benefit *I. livornica* in north Africa, neither can the incidence of African locusts have much effect on *I. lineata* of America. Incidentally, it has yet to be shown that either sub-species ever occurs in forests, or near enough to them to gain by their temporary destruction.

A possible explanation of the coincidence between outbreaks of sub-species in two continents would be that both were subject to a periodicity of the same length, and that the periodicities were in phase with one another. A glance at figs. 1 and 4 will show, however, that there is no regular periodicity.



An example of a somewhat irregular periodicity to which much has been attributed in recent years lies to hand in the sunspot cycle. Sunspot activity can be measured by counting the number of spots each year, and the figures of such counts are available for the whole period covered by the work on *I. livornica*. The number of spots varies from none (1810) to 137 (1837); more general minima being 3 to 5, and maxima 80 to 90. The maxima occur on an average every eleven years, after which there is a fairly gradual fall towards the minimum, followed by a more rapid rise to the next maximum.

There does seem to be some relationship between the outbreaks and sunspot cycle, as shown in fig. 5, which may be expressed in tabular form as follows:

		Total.	Minimum to Maximum.	Maximum.	Maximum to Minimum.	Minimum.
Years		107	32	11	54	10
Outbreaks	To be expected in absence of correlation . .	—	12.3	4.2	20.7	3.8
	Observed . .	41	14	6	20	1
	Difference .	—	+ 1.7	+ 1.8	— 0.7	— 2.8

There have been forty-one outbreaks in the past 107 years, and if there were no connection between outbreaks and sunspots, this proportion should be preserved throughout. The figures predictable on this basis are given in the second line of the table, and in the fourth line the difference is shown between these figures and the actual outbreaks recorded. The number of outbreaks would seem to rise from the minimum towards the maximum, and then to fall away again towards the next minimum, the difference between predicted and observed values being greatest at the year of minimum activity, where the figures are just significant. But the possibility of the apparent connection being due to chance is not precluded; it is worthy of note that some of the most recent and widespread outbreaks (1930-1932) have taken place during a period of rapidly falling activity.

Reference has been made earlier to the possibility of climate influencing the outbreaks of this moth, and rainfall immediately comes to mind as the factor most likely to be effective. While rain is usually supposed to be absent

	Nevada			Utah		Arizona		Colorado		New Mexico	Total	Wet or Dry	lineata outbreaks
	Battle M.	Tonopah	Fallon	Monticello	Moab	Salt Lake City	Yuma	Phoenix	Colorado Springs	Las Animas	Albuquerque or Santa Fe		
1901	-	+	+	+	+	+	+	+	+	+	8	3	W
2	+	+	-	-	-	+	+	+	+	+	5	6	D
3	+	-	-	+	+	+	+	+	+	+	8	3	W
1910	+	+	+	+	-	-	-	-	+	+	7	4	W
11	+	-	-	-	+	-	+	-	-	-	4	7	D
12	+	-	-	-	-	-	+	-	-	-	4	7	D
13	-	+	+	+	+	-	-	-	-	-	5	6	D
14	+	+	+	+	+	+	-	-	+	+	9	2	W
15	+	+	+	+	+	-	+	+	+	+	10	1	W
16	-	+	+	+	+	-	+	+	+	+	7	4	W
17	-	+	-	-	+	+	-	+	-	-	6	5	W
18	-	-	-	-	-	-	-	-	-	-	11		D
19	-	+	+	-	+	-	+	-	+	+	6	5	W
1920	-	-	-	-	-	+	+	+	+	+	4	7	D
1	-	+	+	+	-	+	-	-	-	-	5	6	D
2	+	+	+	+	+	+	+	+	+	+	9	2	W
3	-	-	+	+	-	-	-	-	-	-	3	8	D
4	+	-	-	-	-	+	+	+	+	+	6	5	W
5	-	-	+	-	-	-	-	-	-	-	1	10	D
6	+	+	+	+	+	+	+	+	+	+	10	1	W

FIG. 6.

from deserts, it is not entirely unknown, especially on their outskirts, and when it does fall its effects are proportionately great. Unfortunately, very few figures seem to be available for the Old World deserts; it would seem that either there are no weather stations in the Sahara, or else that a rain gauge is not considered a proper part of their equipment. By courtesy of the London Meteorological Office, I have been able to obtain a few figures; most of them come from Egypt, but one or two series are from stations near to the more westerly part of the Sahara, and one is from Tiflis, in Asia Minor; most of the series are comparatively short and much broken. There does not seem to be any correlation between them and the outbreaks of *l. livornica*, but the figures are far too scanty for it to be said with certainty that no correlation exists.

From America, the United States Department of Agriculture Weather Bureau have sent me copies of their climatic summaries for the relevant States. Many of these summaries go back before 1875, the year of the first recorded *l. lineata* outbreak, and from them it has been possible to compile a table showing the departures from the annual mean rainfall in eleven scattered desert States. A portion of this table is illustrated in fig. 6. Since outbreaks tend to occur about June, the year's rainfall has been calculated from June to May, instead of from January to December, the more usual method. Where the stations showing a deficiency in any year outnumber those showing an excess,

it is counted a dry year; where the reverse occurs, it is counted wet. This gives a rough criterion by means of which it is possible to divide the period into years of relatively high and relatively low rainfall, as a basis for comparison with years when outbreaks of *lineata* occurred. The direct comparison of years of outbreak with the rainfall of the previous twelve months does not give a very promising result. There is a slight tendency for outbreaks to follow wet years, but the figures are not significant, and no conclusions can be drawn from them. If, however, the weather for two years preceding the outbreaks be taken into consideration the very interesting result is found that there is a distinct tendency for outbreaks to occur when a wet year has followed a dry year. These results may be summarised as follows :

		Total.	Dry years following dry years.	Dry years following wet years.	Wet years following wet years.	Wet years following dry years.
Years	.	60	22	13	11	14
Outbreaks	To be expected in absence of cor- relation	—	8.4	5.0	4.2	5.4
	Observed	23	8	3	2	10
	Difference	—	— 0.4	— 2.0	— 2.2	+ 4.6

Here, the observed values are below those predicted in every column except the last, where wet years following dry years are shown. The figures are significant, and the chances against them having occurred by accident in this particular proportion are about forty-five to one. The interpretation of these results is by no means easy; indeed, it is difficult to make even an intelligent guess to account for the connection between this particular sequence of weather conditions and the subsequent migrations. One might imagine that the dry year forced the insects out to the edges of the desert area, and that if subsequent wet weather should stimulate breeding and produce over-population, the moths would migrate outwards to cultivated lands as well as inwards towards the desert again. But this is to indulge in pure speculation; the interesting point is that these figures do strengthen the suggestion that *C. lineata*, at least, is a species of desert or semi-desert origin. It is a great pity that the paucity of weather records do not permit us to check it by comparison with the African sub-species.

Acknowledgments.

My thanks are due to a number of people who have helped me with information about the occurrence of *Celerio l. lineata* and *C. lineata livornica*, and on other points which have arisen in the course of the work; to Dr. A. S. Hayt, of the United States Department of Agriculture, for information about the years of abundance of *C. l. lineata* in America; to the many observers of the Insect Immigration Committee, and to all those who have sent me particulars of *C. l. livornica* in private collections in this country. I am also indebted to the Secretary of the Royal Observatory, Greenwich, for data on sunspots, and to the Directors of the United States Department of Agriculture Weather Bureau and the London Meteorological Office for records of rainfall.

In particular my gratitude is due to Mr. W. G. Cochran, of the Rothamsted Statistical Department, for the patience with which he has helped me with the statistical parts of this paper.

References.

During the course of the work, about two thousand volumes have been consulted, and nearly a thousand abstracts have been made. To give a complete list of sources would involve many pages of print, and would probably be of very little practical value. A full index of references is preserved at the Rothamsted Experimental Station, where they may be consulted by anyone interested in the subject.

The chief publications studied were :

The Entomologist.

The Entomologist's Monthly Magazine.

Annales and Bulletin de la Société Entomologique de France.

Annals and Magazine of Natural History.

The Zoologist.

The Review of Applied Entomology.

Horae Societatis Entomologicae Rossicae.

Boletín de la Sociedad Entomológica de España.

Stettiner Entomologische Zeitung.

When an adequate index was present, such publications were taken volume by volume from their commencement. Many, however, had only lists of titles, and in these the method used was to pick a promising title, read the article concerned, and follow up any reference that might be given. A good deal of information was probably lost in this way, but the only alternative was page-to-page search, an impossibly lengthy task. Innumerable local lists of Lepidoptera for all parts of Europe were consulted, and some references were found in standard works, such as Tutt's *British Lepidoptera*, etc. Wherever possible such references were traced to their original sources, but this could not always be done, especially in the case of some of the earlier Russian writers. Some of the sources seem to have been privately circulated local lists in manuscript, and others I have been unable to trace at all.

Summary.

The distribution and outbreaks of the sub-species *Celerio lineata lineata* Fab. in America, and *Celerio lineata livornica* Esp. in the Old World as far as they are known are described. It is suggested that both sub-species originate in semi-desert areas, and this idea is supported in the case of the American sub-species by showing that a correlation exists between outbreaks of the moths and a certain sequence of desert rainfall. No correlation is found between European outbreaks and the rainfall of those North African meteorological stations for which records are available, but this may be due to the paucity of suitable figures.

A full account is given of the occurrence of *C. l. livornica* in Great Britain, and the main European outbreaks are listed. A correlation is given to show that both years of unusual abundance and of absence tend to occur simultaneously in Europe and America, and that the cause of outbreaks must therefore be sought in some factor common to the two continents.

There seems to be some correlation between outbreaks and the sunspot cycle, but the figures are barely significant. The outbreaks tend to occur away from the sunspot minima.

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THE USE OF LOGARITHMS IN THE INTERPRETATION OF CERTAIN ENTOMOLOGICAL PROBLEMS

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(With 4 Text-figures)

IN many experiments involving a comparison of the numbers of insects present under different conditions—for example, the number of insects on alternative host plants, or in field plots under different treatments; the number of insects attracted to different baits or lights, or attracted to these under different conditions—it is frequently necessary to group together the catches in several cases under one set of conditions and to compare them with a number of cases under different conditions. One wishes, in fact, to compare the “average” catch under the two conditions.

In most cases one does this by adding together the numbers obtained under similar conditions and dividing it by the number of cases, thus obtaining an average which is an “arithmetic” mean. It is the purpose of this note, however, to show that in some experiments a more exact interpretation of the results can be obtained by the use of a “geometric” mean instead of an “arithmetic” mean, and indeed that conclusions drawn from the latter may at times be erroneous. The “geometric” mean is most simply obtained by adding together the logarithms of the numbers in question, finding the “arithmetic” mean of these logarithms and reconverting back from this (when necessary) to numbers again.

During the past 3 years we have had working at Rothamsted a light trap for catching insects at night. The number of insects caught per night has varied from zero to 72,000, and the total for the 3 years, March 1933 to March 1936, is over 600,000.

If we compare the “arithmetic” mean of the captures on nights with one set of weather conditions with the “arithmetic” mean of nights with another set of weather conditions a serious error becomes apparent, owing to the very great variability of the numbers and the swamping effect that a single large catch can have on the “arithmetic” mean.

For example, on 1 January 1935 more insects were captured in the trap than in the whole of January, February, March and April of the previous year. Any comparison which includes these two sets of figures is liable to be considerably biased by the one night. Again, the number of noctuid moths caught on the successive nights of the full-moon week in October 1933 was 0, 0, 1, 62, 0, 0, 0. In the corresponding no-moon week the numbers were 2, 4, 0, 0, 10, 3, 3. If we compare the total capture in the full-moon week (63) with that of the no-moon week (22) it is obvious that the former is unduly biased by the single large catch.

Reasoning *a priori* one might expect that similar differences in environment would produce similar percentage increases in a catch rather than similar numerical increases. Thus if the catches were on two nights under one set of conditions 100 and 1000 insects; and if in another set of conditions (for example, a second trap with a more powerful light) the catch on the first night was 200, one might expect the catch on the second night to be 2000 and not 1100, i.e. a similar percentage increase and not the addition of 900 insects to each catch. The addition would be in proportion to the basic catch.

If this reasoning is correct the proper mean to take for the comparison of two series of figures would be the geometric mean and not the arithmetic mean. This, however, would be a cumbersome piece of work if done arithmetically, and exactly the same result can be obtained by using the logarithm of the number caught in each case instead of the actual numbers; then for a series of nights one could use either the sum of the logarithms of the individual nights (not the logarithm of the sum of the numbers), or the average logarithm; or the latter could be reconverted back to the geometric mean by taking the anti-logarithm.

For example, if a series of catches under two different conditions are

Series A	5	15	47	1000	2	8
Series B	4	19	22	99	50	17

the comparison of the total numbers is 1077 : 211, or of the arithmetic mean catch 180 : 35. Series A therefore has the higher average.

The logarithms of the above series (to two decimal places, which has been found sufficient for all practical purposes) are

Series A	0.70	1.18	1.67	3.00	0.30	0.90
Series B	0.60	1.28	1.34	2.00	1.70	1.23

So the comparison of the logarithmic sum is 7.75 : 8.15, the comparison of the mean logarithm is 1.29 : 1.36, and the comparison of the geometric mean (anti-logarithm of above) is 19.6 : 22.8. It will be seen that the

large catch of 1000 on one night of the first series no longer swamps the proportion, and series B has a higher mean than series A.

A complication ensues if any value in the series is zero, for the logarithm of zero is minus infinity and the geometric mean of any series containing a zero is itself zero. It has been found possible in these cases to add a unit to all values in the series before taking logarithms, i.e. to deal with $\log (n+1)$ instead of $\log n$. If this is done however it is necessary to subtract the unit from the final result when it is reconverted back into number from logarithms.

If this system is used it is found that the sum of $\log (n+1)$ for the values quoted above for the full-moon week captures of Noctuidae become 2.10 while that for the no-moon week is 3.89. Thus the geometric mean for the no-moon week is higher than the full-moon week.

Another way in which the use of the logarithm is found to be more suitable is in the distribution of departures from a mean. If an arithmetic mean value be taken for a series of captures on the number basis and if each day's value is expressed as a departure from the mean, then in the case of actual numbers (see examples 3 and 4 below) the values are made up of a large number of small negative departures and a small number of large positive departures which give a skew curve which does not lend itself to treatment by the normal formulae of standard deviation, etc. If on the other hand the departures of the logarithms of $n+1$ from a mean logarithm are used, the number of values on either side of the mean is almost equal and their distribution near to normal.

In one case (see example 3 below) the square root of each number was taken as a test and this was found to give a skew distribution, less asymmetrical than number curve but definitely not so good as the logarithm distribution.

It is thought that the best way of explaining the methods and results more fully would be to give a few examples from actual calculations that have occurred in the analysis of the trap records.

Example 1. A comparison of the catches in a single trap on alternate nights

There is no reason to suppose that with the same trap in the same spot there should be any consistent difference between captures on two series of alternate nights. Such differences as occur are due to accidental alterations of temperature, wind and other weather conditions, superimposed upon which is the experimental error.

To test the error that might occur from these causes in a long series of nights, the captures in the light trap were added together in two series: (a) those on the odd nights of the year (1, 3, 5, etc.), and (b) those caught on the even nights (2, 4, 6).

Table I shows the results when the numbers themselves are summed; when the square roots of the numbers are summed; and when the $\log(n+1)$ are summed for each of the three years 1933-5. The number of nights on which the trap was working was 306 in 1933 (March to December), 364 in 1934 and 358 in 1935.

Table I

	1933 (9 months)		1934		1935		All 3 years	
	Odd	Even	Odd	Even	Odd	Even	Odd	Even
Σn	61,368	47,385	56,689	40,260	243,556	160,468	361,613	248,113
Ratio n	129	100	141	100	152	100	145	100
$\Sigma \sqrt{n}$	2,131	1,975	2,198	1,897	—	—	—	—
Ratio n	117	100	135	100	—	—	—	—
$\Sigma \log(n+1)$	266.62	265.70	301.73	288.39	322.25	327.77	890.60	881.86
Ratio n	101.4	100	118.5	100	100	107.4	104.2	100

It will be seen that by the accidental distribution of weather conditions and experimental error the odd nights in each year, when summed on a number basis, have differed very considerably from the even nights. The total catches on the odd nights were greater by 29 per cent in 1933, by 41 per cent in 1934, and by 52 per cent in 1935: with a total difference on all 3 years (including over 1000 nights) of 45 per cent. Thus if a different trap or a different intensity of light had been used for one of the two series a difference of 45 per cent after 3 years would have demonstrated no real difference in efficiency.

When the \sqrt{n} was used as a basis of summation (this was not done for the third year as no advantage seemed to be gained) the ratios between the mean catches were lower but still large, 17 per cent in 1933 and 35 per cent in 1934.

When $\log(n+1)$ was used the difference was reduced to 1.4 per cent in 1933, to 18.5 per cent for 1934, and to 7.4 per cent in favour of the even nights in 1935. The difference in the total captures on the odd and even series in 1935 was about 83,000 insects but of these 72,000 occurred on one night, so that the great effect produced by the use of logarithms is not so unexpected.

For the 3 years together the use of logarithms have reduced the difference to 4.2 per cent from the 45 per cent difference in actual numbers.

Example 2. *A comparison of the catches of Noctuidae in weeks of full-moon and of no-moon*

Table II shows the captures of Noctuidae in the full-moon and no-moon weeks of six summer months in each of the 3 years 1933-5. The totals are based first on a sum of the actual numbers caught (n) and secondly on a sum of $\log(n+1)$. The larger number in each pair is in heavy type (Williams, 1936).

Table II

Table II									All
		May	June	July	Aug.	Sept.	Oct.	Total	3 years
Numbers $\Sigma (n)$									
1933	Full	15	55	58	64	29	63	284	1242 2859
	No	19	95	73	72	76	22	363	
1934	Full	2	52	37	69	61	1	222	
	No	25	56	140	76	204	71	575	
1935	Full	2	15	477	168	64	10	736	
	No	23	179	917	385	267	133	1904	
Logarithms $\Sigma \log (n+1)$									
1933	Full	2.76	7.51	6.49	6.03	4.25	2.10	29.14	83.41 133.20
	No	3.59	7.36	7.14	7.14	7.34	3.89	36.46	
1934	Full	0.60	4.20	5.31	6.63	5.77	0.30	22.81	
	No	3.64	5.86	9.01	7.11	10.20	6.95	42.77	
1935	Full	0.60	2.75	10.87	8.99	6.32	1.93	31.46	
	No	3.49	7.93	13.86	11.70	9.92	7.07	53.97	

In each case 17 out of the 18 weeks give values in favour of no-moon, indicating that there is undoubtedly a consistent difference between the full- and no-moon weeks.

With the numbers the mean difference per week between the two is 87.6 with a standard deviation of ± 27.3 . This gives a " t " test (that is, the mean difference divided by the standard deviation) of 3.2.

On the logarithmic basis the mean difference per week is 2.77 with a standard deviation of ± 0.44 which gives $t=6.3$. Thus, as t is a measure of the significance of the results, the use of the logarithms has given a result of very much higher significance than the use of numbers. The mean catch per night calculated from the numbers (that is arithmetic mean) is 9.9 insects for the full-moon nights and 22.7 for the no-moon nights or a ratio of 100 to 229. The geometric means (obtained by reconvertng the mean logarithm per night back to an anti-logarithm and subtracting one) is 3.59 insects for the full-moon nights and 10.40 for the no-moon nights or a ratio of 100 to 289. It is therefore seen that as the differences were consistent they have not been reduced (but actually increased) by the use of logarithms, in direct comparison with the results obtained in the first example where non-consistent differences are very much reduced.

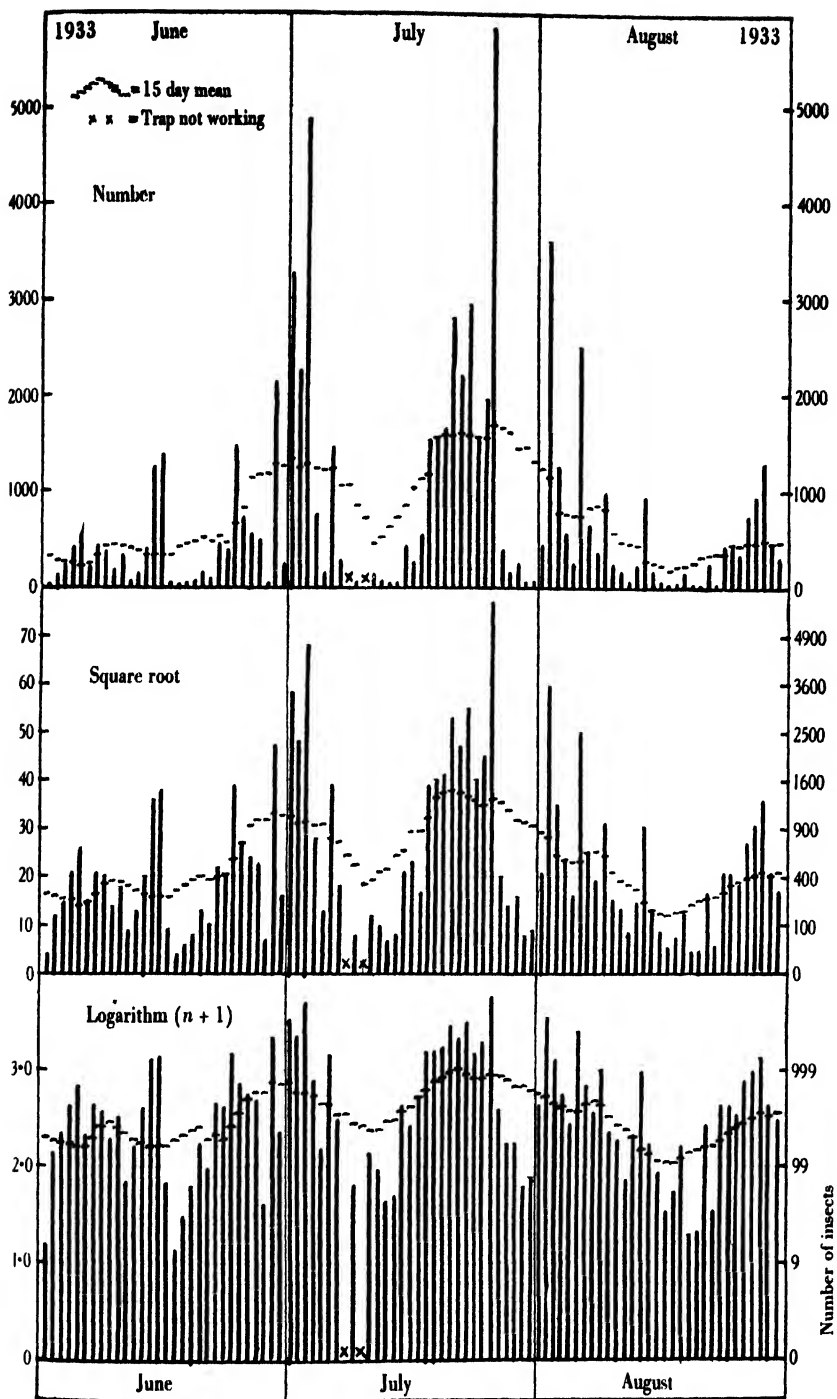


Fig. 1.

*Example 3. Captures of all insects in June to August 1933
and the departures from a mean*

Fig. 1 shows the captures day by day for the months of June, July and August 1933 of all insects, first on a number basis, then on the square root, and finally on the basis of $\log n + 1$; with a running 15-day mean of the values in each case.

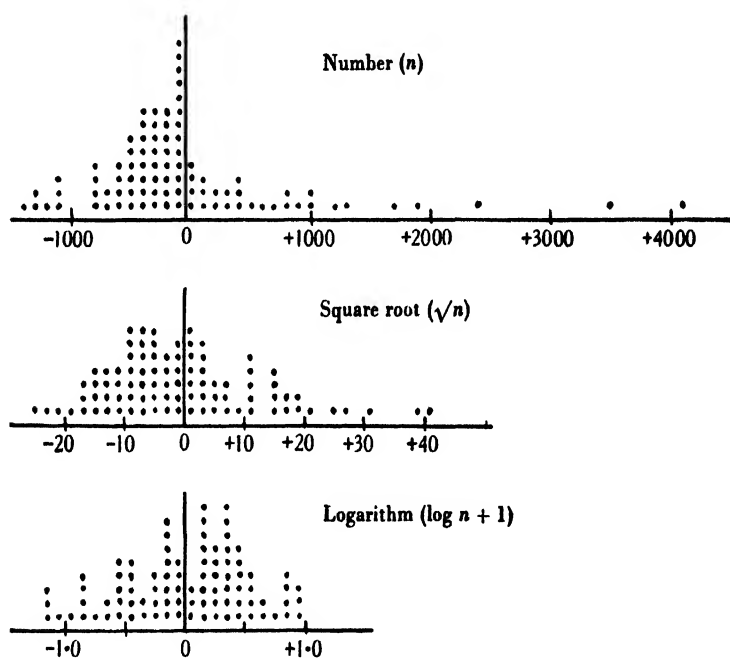


Fig. 2.

Fig. 2 shows the distribution of the departures from the mean in each of the three cases. It will be seen that on the number basis the departures consist of a larger number (60) of smaller negative departures and a much smaller number (29) of larger positive departures, giving a skew distribution.

The square roots give a less skew curve with 51 negative and 39 positive departures, the latter having nearly twice the range of the former. The logarithm gives 41 negative and 47 positive departures with almost the same range.

Example 4. Time of flight during the night of all Diptera in the years 1933, 1934 and 1935, and month by month in 1935-6

The light trap which is in use at Rothamsted is fitted with a mechanism so that eight killing bottles pass under the light in succession each night. The timing is so arranged that each bottle contains the insects caught in one-eighth of the night, starting from half an hour after sunset and ending half an hour before sunrise. Four of the bottles are before, and four after midnight.

If over any series of nights the captures in bottle 1 or bottle 2 etc. are added together, a general measure of the abundance of insects caught at that time of the night can be obtained, and hence, from all the bottles, the distribution of the captures during the night.

Table III shows the number of Diptera caught in each period of the night in each month of 1935-6 on the basis of the sum of numbers and the sum of $\log(n+1)$. The maxima in each month are in heavy type and the minima in italics.

Table III

Period of night ...	1	2	3	4	5	6	7	8
	Number							
1935 Mar.	361	214	140	139	117	166	107	72
Apr.	187	109	84	78	38	34	9	14
May	2,204	2,204	807	1,081	511	443	<i>206</i>	293
June	<i>4,190</i>	16,859	15,034	14,700	14,319	7,169	8,751	4,430
July	5,893	12,568	14,489	23,356	9,254	8,652	7,112	<i>5,771</i>
Aug.	5,746	6,592	7,958	6,950	5,015	3,717	6,599	<i>3,166</i>
Sept.	2,580	1,922	1,277	1,641	2,356	2,334	1,091	<i>1,018</i>
Oct.	3,396	1,462	954	1,176	1,552	787	<i>574</i>	1,354
Nov.	933	497	238	316	1,135	1,382	2,601	1,456
Dec.	113	81	69	63	79	67	189	128
1936 Jan.	198	233	413	111	201	131	112	72
Feb.	49	10	62	85	100	6	10	5
1935-36 Total	25,850	42,751	41,525	49,696	34,677	24,888	27,361	<i>17,779</i>
1933-34 "	12,564	13,184	11,756	10,304	10,810	9,341	7,798	<i>6,031</i>
1934-35 "	11,193	8,221	6,571	6,657	8,224	6,956	<i>5,433</i>	7 647
	Logarithm ($n+1$)							
1935 Mar.	22.38	15.90	13.30	10.79	9.10	9.71	10.60	<i>8.04</i>
Apr.	14.50	11.44	9.65	8.43	6.06	5.67	2.28	2.95
May	29.76	26.15	22.84	23.44	19.00	18.20	<i>15.07</i>	18.44
June	42.99	49.42	43.41	40.42	41.12	<i>34.63</i>	37.61	40.01
July	58.20	62.55	62.51	59.33	55.45	53.22	49.39	<i>45.37</i>
Aug.	59.49	57.31	55.58	52.40	49.35	<i>45.44</i>	46.63	45.57
Sept.	46.22	40.29	35.15	33.46	35.96	30.70	<i>26.57</i>	30.31
Oct.	27.58	22.68	<i>22.44</i>	25.55	24.78	24.48	23.75	<i>22.46</i>
Nov.	31.56	19.53	<i>15.65</i>	18.17	17.39	16.47	21.39	20.40
Dec.	12.48	7.04	7.30	8.35	7.99	<i>5.69</i>	8.47	11.01
1936 Jan.	12.06	6.72	10.33	8.41	6.14	<i>5.32</i>	6.17	6.04
Feb.	5.04	2.28	3.02	2.23	3.18	<i>0.85</i>	1.82	1.38
1935-36 Total	362.26	321.3	301.2	291.0	275.5	250.4	<i>249.8</i>	252.0
1933-34 "	280.9	264.4	242.1	226.3	216.8	219.9	194.3	<i>187.2</i>
1934-35 "	311.8	247.7	219.2	209.6	199.0	184.0	<i>164.5</i>	190.2

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It will be seen that the logarithmic results are much more regular than the number results. In the latter case the maximum is in six months in the first period, twice in the second, twice in the third, once in the

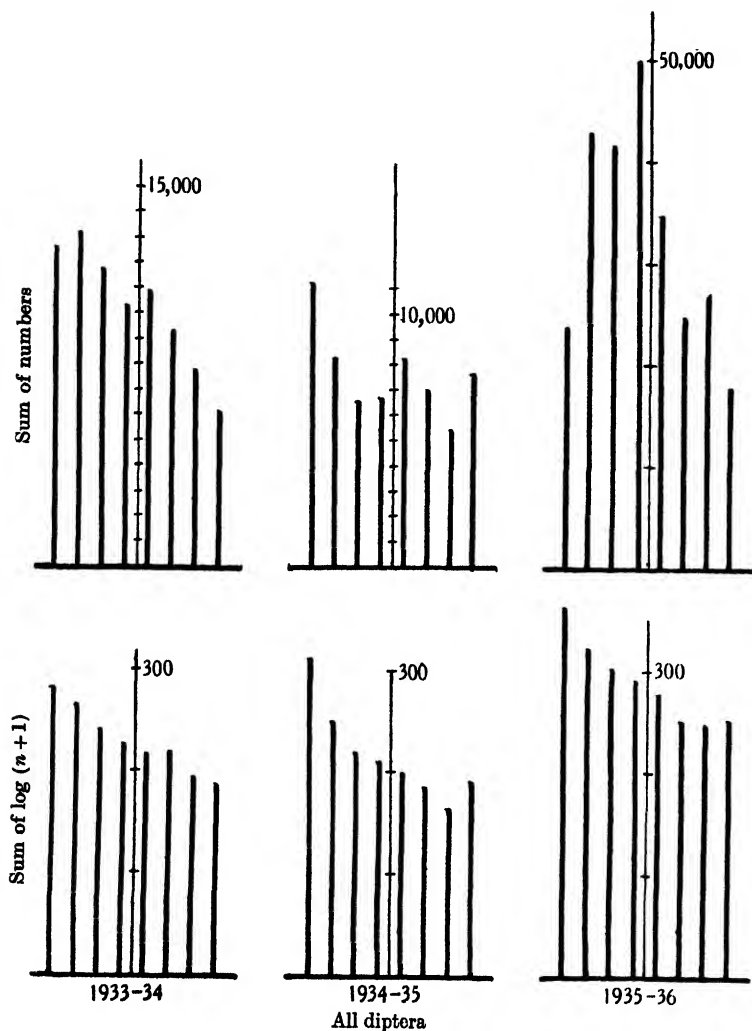


Fig. 3.

fourth and twice in the seventh. On the logarithm the maximum is in the first period in ten months and in the second in two.

When the annual totals are compared (Table III and Fig. 3) the numbers show a maximum once in the first period, once in the second and once in the third. The logarithm shows all 3 years with the maximum

in the first period. The reason that the sum of numbers generally gives a maximum flight later in the night than the logarithmic sum is that nights with exceptionally high catches tend to be nights in which the activity is late. This can be tested by separating the nights in each month into classes based on whether the catches are above or below normal. When this is done both numbers and logarithms show similar

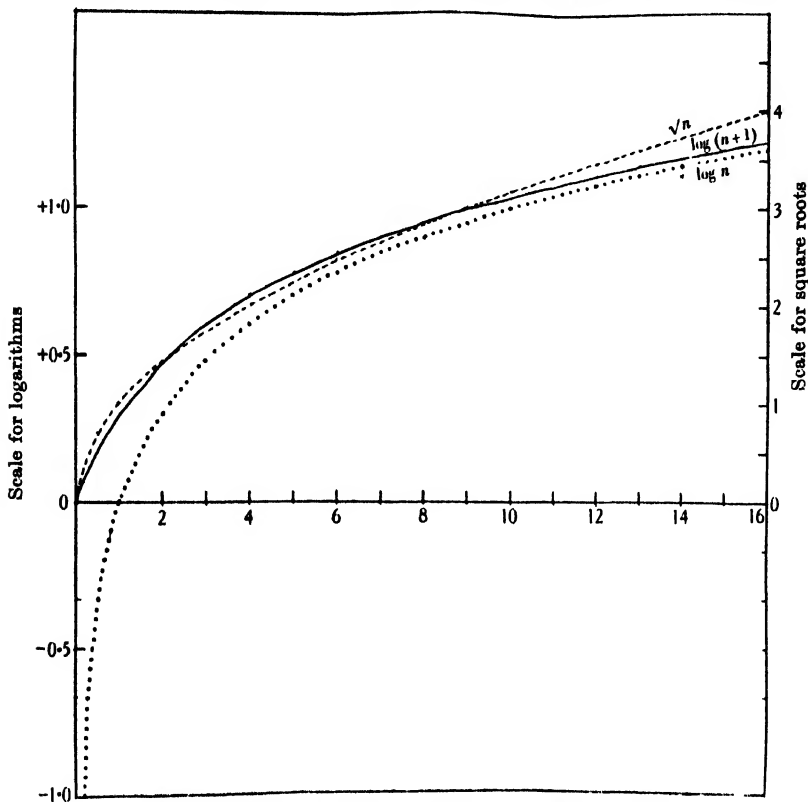


Fig. 4. ——— $\log(n+1)$; ---- square root n ; $\log n$.

differences, indicating that the effect is real and consistent (Williams, 1935, p. 533).

Note. Mr W. Yates of the Statistical Department at Rothamsted Experimental Station has drawn my attention to the fact that the curve of the relation $y = \log(n+1)$ closely approximates to the curve $y = \frac{1}{3}\sqrt{n}$ for low numbers, being identical when $n=0$, 1.8 and 9. At values of n above 10 it gradually departs from the square-root curve and approaches more and more closely the curve $y = \log n$ from which it is practically indistinguishable (in the second decimal place) at values above 100.

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Fig. 4 shows the relations graphically. The result is that by using the value $\log (n+1)$ in analysis as above, we are substantially using a value proportional to \sqrt{n} for low numbers and one proportional to $\log n$ for high numbers.

SUMMARY

Evidence is brought forward that in comparing the numbers of insects caught under varying conditions, with particular reference to captures in a light trap, more consistent results are obtained if the geometric means are compared than by the use of arithmetic means. This is most conveniently done by summing the logarithms of the numbers instead of the numbers themselves. If any of the numbers in the series is zero it has been found practical to add one unit to all the captures in the series and so deal with $\log (n+1)$ instead of $\log n$.

The use of the logarithms prevents the swamping of the results in a series of observations by very high numbers on a single night. It also gives a more normal distribution of departures from a mean. As a result of the latter it is possible to apply the statistical formulae for standard deviation etc. which are not applicable to the skew curve obtained by the use of the departures of the numbers themselves from an arithmetic mean.

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A NEW APPARATUS FOR SEPARATING INSECTS AND OTHER ARTHROPODS FROM THE SOIL

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(With Plate XXXIX and 2 Text-figures)

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I. REVIEW OF METHODS PREVIOUSLY USED

IN the course of some investigations now being carried on at Rothamsted on the action of soil fumigants, the ability to determine rapidly the density and nature of the soil population became essential. A scrutiny of the literature (1, 2, 3, 4, 9, 10, 12 and 13) showed that in all investigations on the insect fauna of the soil their quick separation, alive and undamaged, was a matter of great difficulty and there was no method that would quite meet our requirements.

The original primitive procedure was to crumble the soil by hand and examine it in small quantities after spreading it out on sheets of paper. With a soil containing much clay it is impossible to get it in a condition friable enough to crumble, without losing many of the smaller insects such as Collembola, and dipterous larvae, both on account of their activity in escaping and by their death on drying. As a result there is little doubt that the numbers recorded are considerably lower than the truth.

The various improvements on this primitive method may be placed in three main classes:

- (1) Voluntary movement of fauna from the soil.
 - (a) Attraction to warmth.
 - (b) Attraction to warmth aided by repulsion from light.
- (2) Separation of fauna from the soil by sieving.
 - (a) Straight sieving of soil in a semi-dry condition.
 - (b) Washing the soil through sieves with or without shaking.
- (3) Separation of fauna from the soil by flotation in water.
 - (a) Without preliminary sieving.
 - (b) On the residue after washing the soil through sieves.

(1) *Voluntary movement of fauna from the soil*

(a) *Attraction to warmth.*

Berlese⁽²⁰⁾ in 1905 devised an interesting piece of apparatus for collecting minute insects from the soil, moss and similar materials. The apparatus consists of a double-walled metal funnel with a fine-mesh sieve across the top and a tube leading to a small bottle at the bottom. The space between the two walls of the funnel is nearly filled with water which is kept warm. The material to be examined is spread out on the sieve, when the animals in it move downward through the sieve towards the warmth and away from the light into the bottle.

According to Williams⁽¹⁹⁾ the greater part of the catch will come through in the first 24 hours.

(b) *Attraction to warmth aided by repulsion from light.*

Trägårdh^(17, 18) made experiments with a modification of the Berlese funnel as improved by Tullgren in 1917. The soil or other material on the sieve was illuminated and heated from above by an electric lamp. The action of the lamp was twofold; one direct, resulting in the rapid movement downward of those animals that react negatively towards the light; and the more slow indirect action of the light and heat, causing a drying out of the upper layers of the material resulting in a movement of the fauna to the damper lower layers.

(2) *Separation of fauna from the soil by sieving*

(a) *Straight sieving of dry soil.*

Lane⁽⁸⁾ in 1928, in order to facilitate the investigation of subterranean insects, particularly wireworms, used a soil sifting machine. The semi-dry soil was passed through screens with meshes of increasing fineness. He

found that a screen of 12 meshes to the linear inch was sufficient to retain bigger larvae; while the smaller larvae and eggs of most of the injurious Elateridae indigenous to the North-West Pacific area of the United States could be separated from the soil by a 40-mesh screen.

(b) *Washing the soil through sieves with or without shaking.*

A most important step forward was made by Morris⁽¹¹⁾ when he introduced a method whereby the soil insects and other arthropods were separated by washing and stirring the soil with a strong stream of water through a series of sieves. The material left on the various sieves was then examined separately. This method gave higher numbers for the faunal population, but the quantity of soil to be examined on the various sieves was still a large fraction of the sample taken.

Morris's apparatus comprised an outer case of galvanized iron supporting three sieves of different mesh, that with the largest mesh being on the top, the intermediate size in the middle and the smallest below. A lead pipe fitted with a large spray nozzle was clamped to the upper edge of the case and was connected at the other end with a water supply; there was also an outlet at the bottom of the case. The soil to be examined was placed in the upper sieve, the water turned on and the soil thus washed out was divided into three lots, the finest particles being carried away with the waste water and the insects being retained on the various sieves according to their size.

Using Morris's method, with a 12-lb. sample of Rothamsted soil the washing occupies 50 min. to 1 hour and the subsequent examination several days.

The disadvantages of this method are:

(1) The long time and labour entailed in picking the insects off the sieves and separating them from the sandy residue.

(2) The insects are badly damaged by the agitation and friction with the stones.

(3) The constant attention required.

(4) The general uncleanness of the process which makes it undesirable to work in an ordinary laboratory.

(5) The impossibility of using the apparatus for the large number of samples required by an experiment designed in accordance with modern statistical procedure.

Shirek⁽¹⁴⁾ tried a modification of Morris's method for separating eggs and young larvae of wireworms. He designed an apparatus consisting of a wooden rack to hold a series of sieves of 10-, 30-, 40- and 50-mesh,

a hose equipped with an adjustable nozzle, and a small funnel with an interchangeable screen bottom for collecting the residue from various pans after washing. The 40-mesh screen retained practically all the eggs; while the newly hatched larvae were caught by the 50-mesh screen.

Thiem⁽¹⁵⁾ also constructed an interchangeable soil sieve, similar to that of Shirck, and used it successfully for separating the pupae of *Rhagoletis cerasi* L. from the soil. It consisted of a tripod supporting a funnel over which were placed four close-fitting sieves one above the other. Each sieve had an interchangeable bottom consisting of a brass screen disc soldered to the metal ring.

(3) *Separation of fauna from the soil by flotation in water*

(a) *Flotation without preliminary sieving.*

Daniels⁽⁵⁾ devised a method which proved very satisfactory in North Eastern Colorado for obtaining the larvae and pupae of the potato flea beetle (*Epitrix cucumeris* Harris) in potato fields. The larvae and pupae, which were too delicate to withstand the ordinary method of sifting and washing, were floated to the surface by agitating the soil in shallow galvanized pans with water which was subsequently poured through brass screens of increasing fineness. The soil was allowed several minutes to settle so as to eliminate clogging of the screen with mud when the liquid was poured off the sample. The rubbish with the pupae and larvae were caught on a 1/20-in. screen, but the majority of the larvae were found on the 1/40-in. screen.

(b) *Flotation after sieving.*

Mabyn Thompson⁽¹⁵⁾ at Aberystwyth after sifting the soil for a preliminary examination, took small portions and washed them through a sieve of about 0.75 mm. mesh, shaking at the same time. The residue on the sieve was then stirred with water in a dish and small organisms would float on the surface of the water. The water was poured off and the process repeated with fresh water until nothing more was found. Edwards⁽⁶⁾ at Aberystwyth modified Thompson's procedure by using a series of three sieves 3.5 mm., 1.5 mm. and 50 meshes to the linear inch respectively.

II. DESCRIPTION OF NEW METHOD

In view of the inefficiency of the existing methods and their unsuitability for modern demands a new system of separation became necessary.

The problem was attacked in two ways:

(a) Direct flotation.

(b) Flotation by making use of the surface relationships of the insects and a liquid medium as used in the separation of minerals.

Only the first method has been developed to a state of efficiency and forms the subject of the present paper.

(1) *Preliminary trials*

The density of typical soil insects in various physiological states was determined and it was found that in no case was it higher than 1.1, even when the insect was contracted.

The next step was the selection of a liquid of the required density which would be non-toxic to the insects when the latter were immersed in it for a reasonable period, say 20–30 min. The liquid must be cheap and easily handled, and have no dispersive action on the clay in the soil.

After sundry trials a solution of magnesium sulphate in water was selected; this has the additional advantage of flocculating the clay, but some other material may be found to be equally or more suitable. Commercial calcium chloride can be used, but it precipitates the “hardness” of the tap water and the clear solution must then be decanted from the precipitate after standing. Moreover, it is more unpleasant to work with than magnesium sulphate. Water alone could be used if only Collembola, mites and other organisms with a low density were required, but in a clay soil a complete separation from the mud would not be possible.

(2) *Principle of the method*

The soil is stirred up gently with a solution of magnesium sulphate¹ (sp. gr. 1.11) in a metal cylinder and a stream of very fine air bubbles is passed up through the mixture. The air bubbles help to free the insects quickly from the soil and these rise to the top of the liquid in a froth mixed with organic debris, most of which, however, is held back by a coarse sieve placed just below the surface. The level of the liquid in the cylinder is raised by the gradual inflow of magnesium sulphate by gravity from a reservoir.

The top of the cylinder is fitted with a conical head culminating in a small rectangular opening. The froth collects in this opening and overflows down a chute into a sedimentation tank full of the magnesium sulphate solution. Any soil coming over with the froth is deposited here while the froth with its content of soil animals sweeps over the surface of the liquid on to a Buchner funnel fitted with two filter papers, No. 1 Whatman underneath, and No. 29, a black paper, on top. The liquid is

¹ Approximately 25 % solution of the commercial crystallized salt (Bath salts).

filtered off and the debris given two quick washings with water. The bigger insects can be seen with the naked eye and are picked up with forceps but for smaller insects the filter paper must be cut into strips and examined under a binocular microscope. The apparatus as at present in use will take 7 lb. of soil, but works better with a smaller quantity, 5-6 lb. Once the soil is added the process of separation is almost automatic, so it would be possible to have several machines in use at the same time or one machine in use while the others were being cleaned. The limiting factor is of course the time necessary for the full examination of the debris. The quantity of residue to be examined is less than 1 per cent of the original soil, whereas with Morris's method and its modifications the quantity may be from 30 to 60 per cent or more.

(3) *Description of the apparatus*

The apparatus (Pl. XXXIX, fig. 1 and Text-fig. 1) consists of:

- (a) A cylinder in which the soil is mixed with the liquid.
- (b) A conical head fitted to the top of the cylinder with a watertight connexion.
- (c) A combined stirrer and air bubbler supporting two sieves.
- (d) A stirring mechanism.
- (e) An air pump and (e_1) manometer.
- (f) A small electric motor for stirring.
- (g) A soil sedimentation tank.
- (h) A glass reservoir containing the solution.
- (i) A Buchner funnel and (j) filter flask.

(a) *The mixing cylinder* (Pl. XXXIX, fig. 1, a and Text-fig. 1, a).

This is made of galvanized sheet iron. It is $8\frac{1}{4}$ in. in diameter and 11 in. high; it has a rim $1\frac{1}{2}$ in. wide soldered on to the bottom to raise it sufficiently to allow clearance for the discharge outlet. This outlet is in the form of a "Honey-Gate" (1) and can be purchased from makers of bee-keepers' appliances. The outlet is fitted on the bottom of the cylinder and projects through the lower rim. By means of the "Honey-Gate" a quick discharge of the contents of the cylinder is obtained.

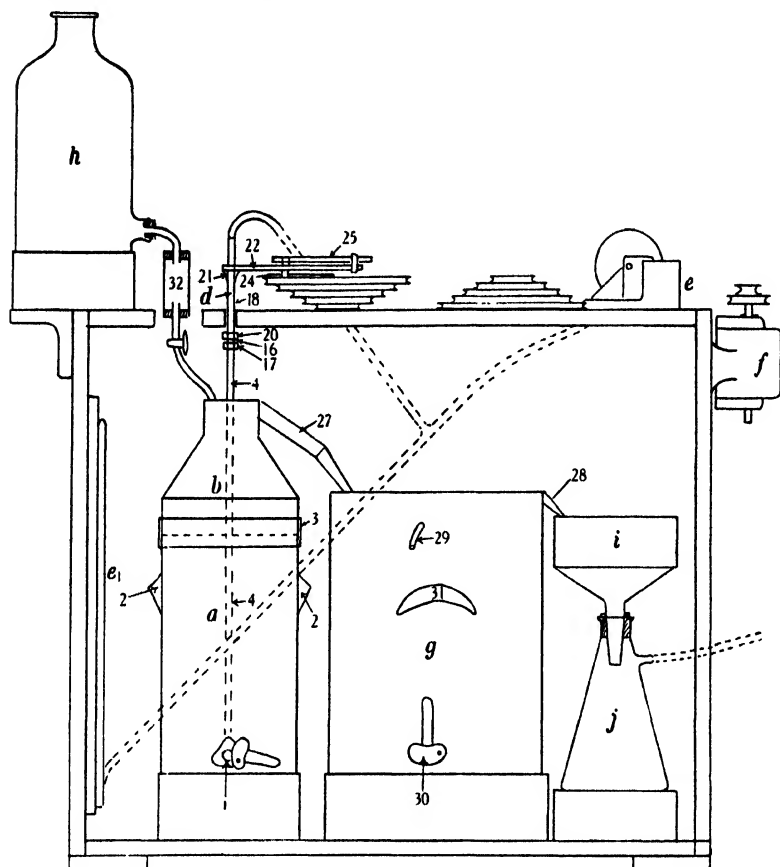
Two handles (2) are soldered opposite each other at right angles to the honey gate. In the centre of the bottom inside is soldered a small piece of brass plate with a hole to act as a bearing for the lower end of the stirrer.

(b) *The conical head* (Pl. XXXIX, fig. 1, b and Text-fig. 1, b).

This is made of galvanized sheet iron. A rim $1\frac{1}{2}$ in. wide fits over the upper rim of the cylinder. The conical portion of the head is 4 in.

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in height and culminates in a rectangle 2 in. high, $4\frac{1}{2}$ in. long and 3 in. in width open at the top.



Text-fig. 1. Diagrammatic representation of the whole apparatus mounted in the wooden frame. For explanation of lettering see text.

Key to numerals used in Text-figs. 1 and 2

1, discharge outlet of cylinder; 2, handles; 3, rubber ring; 4, central tube of hollow stirrer; 5, hexagonal box of stirrer; 6, air tubes; 7, air outlet; 8, brass caps; 9, brass wire; 10, clip for baffles; 11, rubber stirring baffles; 12, bearing point; 16, threaded collar; 17, hexagonal nut; 18, brass tube connexion; 19, flanged tube; 20, hexagonal back nut; 21, brass boss; 22, iron strap; 23, brass pin; 24, crank; 25, connecting arm; 26, baffle plates; 27, chute; 28, lip; 29, over-flow pipe; 30, discharge outlet of tank; 31, handles; 32, tap funnel.

The watertight connexion between *a* and *b* is made thus: the upper rim of the cylinder and the rim of the conical head are each covered with a wide rubber ring and fixed in position with paint. In addition,

there is another ring 3 in. wide (3) over the rubber ring on the rim of the cylinder, fixed in position by means of adhesive tape and copper wire. The upper half of this is rolled back and when the conical head is placed in position the ring is pulled over the rubber on the rim of the conical head and makes a perfectly watertight joint.

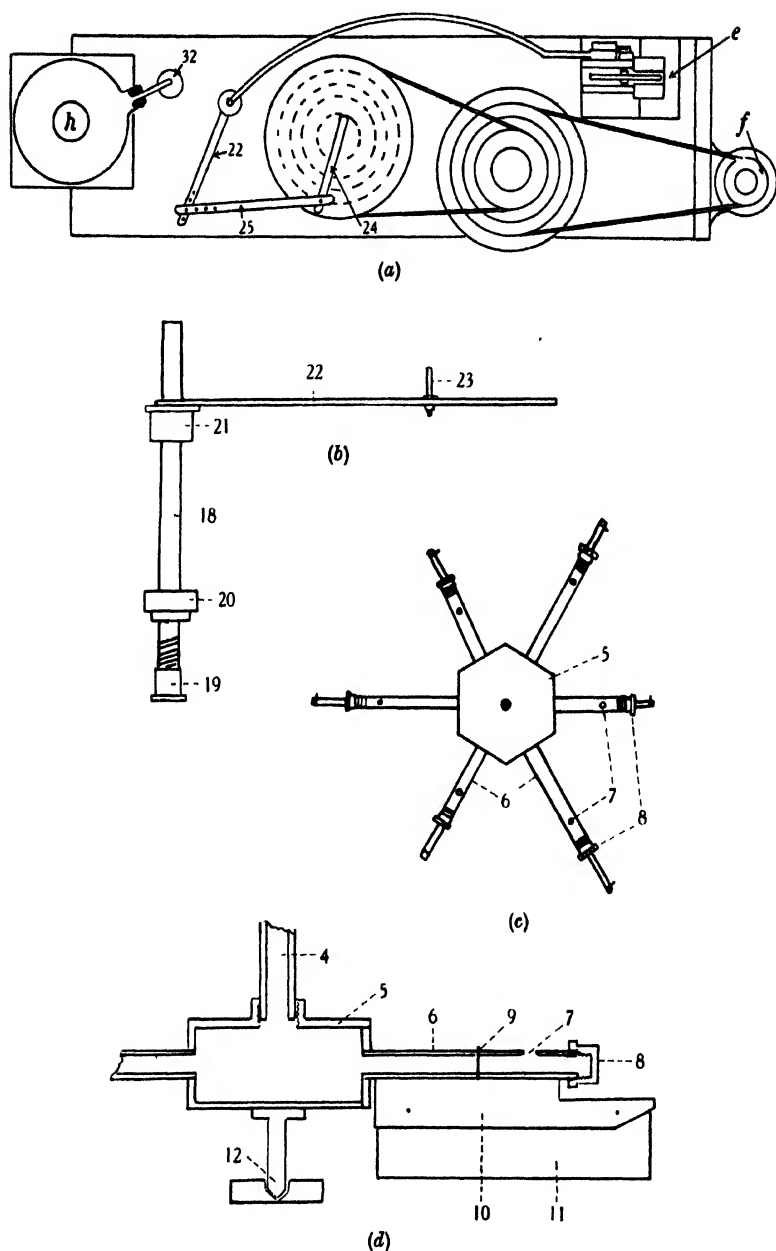
Note. An improved mixing cylinder and conical head (Pl. XXXIX, fig. 2). The cylinder is fitted with a narrow flange $1\frac{1}{2}$ in. wide. On this flange is fixed a soft rubber washer $\frac{1}{8}$ in. thick. The conical head is provided with a similar flange and rubber washer. Six bolts are fixed to the flange on the cylinder and pass through holes in the upper flange. Over each bolt is fitted a loose strip of iron 1 in. wide and 3 in. long with a hole in the middle. The flanges may then be tightened together to make a waterproof joint by means of butterfly nuts.

The connexion between the conical head and cylinder could be made with flanges and hinged bolts such as are used with autoclaves, but in that case very much heavier flanges would be required which would add considerably to the cost of the apparatus.

(c) *Combined stirrer and air bubbler* (Pl. XXXIX, fig. 3 and Text-fig. 2, (c), (d) and f).

A brass tube, $\frac{3}{8}$ in. in diameter and $23\frac{1}{2}$ in. long (4), is screwed at its lower end into a hexagonal brass box (5) $2\frac{1}{2}$ in. in diameter with faces $\frac{7}{8}$ in. deep. In the centre of each face of the hexagon a brass tube (6) $\frac{1}{16}$ in. in diameter is screwed and soldered. These tubes are alternately short and long, the short ones projecting $1\frac{3}{4}$ in. and the long ones $2\frac{1}{2}$ in. from the faces of the box.

A hole (7) $\frac{1}{8}$ in. in diameter is bored in the upper surface of each tube about $\frac{3}{4}$ in. from the outer end which is threaded and closed with a small brass cap (8). A piece of strong brass wire (9) is inserted through each tube half-way between the hole and base of the tube, and soldered in position. To the lower portion of each tube is soldered a length of channel brass (10) 3 in. long and $\frac{1}{2}$ in. wide, cut away to clear the brass caps. (If the side tubes were threaded internally, brass plugs could be used instead of the caps and this cutting away would be unnecessary.) The channel brass forms a clip holding narrow pieces of thick rubber (11) (3 in. long and $\frac{3}{4}$ in. wide) which are kept in position by two lengths of copper wire or by rivets passing through holes in the brass and rubber. The rubber strips form the stirring baffles. A piece of $\frac{5}{16}$ in. brass rod (12), $\frac{7}{8}$ in. long and pointed is screwed into a small hexagonal nut soldered on to the centre of the lower surface of the hexagonal box to act as a



Text-fig. 2. *a*, plan of reducing gears and stirring mechanism; *b*, elevation of stirring mechanism connexion; *c*, plan of base of stirrer and air bubbler; *d*, cross-section through base of stirrer.

bearing point. After removing the caps, the side tubes are packed tightly with cotton wool pressing against the cross-wires, and the caps replaced.

When air is pumped through the centre tube it passes through these cotton-wool pads and emerges as a stream of fine bubbles. Some little practice is needed in order to get the packing even in all the six tubes.

Experiments are being made with sintered glass and with a special type of porous stone as a substitute for the cotton wool.

The sieves (Pl. XXXIX, fig. 3). The bottom sieve is 8 in. in diameter—3 meshes to the linear inch, with a vertical rim $1\frac{1}{4}$ in. in height. The sieve is fitted with a central collar connected to the rim by means of two lengths of iron rod. The collar is bored and threaded to take a butterfly screw to fix the sieve in any desired position. The upper sieve is of the same mesh as the lower, but slightly smaller in diameter. It is dome-shaped with the convex side upwards. It is fitted with a collar and butterfly screw so that it can be fixed in a high position, out of the way, while the soil is being put into the cylinder. The lower position of the sieve is fixed by a fork made of brass with its two prongs pointing upwards. The cross bar of the sieve sits in between the prongs when the sieve is lowered. This position is such that the top of the sieve is just below the top of the cylinder.

At the top of the central tube a threaded collar (16) is fixed, $\frac{1}{2}$ in. long and $\frac{5}{8}$ in. in diameter with a hexagonal nut (17) 1 in. in diameter below it.

(d) *The stirring mechanism* (Pl. XXXIX, fig. 1, d, Text-fig. 1, d and Text-fig. 2, a and b).

A brass tube $7\frac{1}{2}$ in. (18) long similar to that used for the central tube of (c) is fitted at the lower end with a wide piece of brass tube $\frac{3}{4}$ in. long with a narrow flange (19) at the lower end screwed on and soldered in position. A hexagonal back nut (20) is a loose fit over the tube and is retained by the flange. This nut is threaded internally to engage with the threaded collar (16) of the stirrer, a lead washer being used to secure an air-tight connexion when the two hexagonal nuts are tightened with spanners.

A brass boss (21) $\frac{1}{2}$ in. wide is slipped over the tube $1\frac{3}{4}$ in. from the top and soldered in position; on this is soldered a piece of iron strip (22) $\frac{1}{8}$ in. thick, 8 in. long and $\frac{3}{4}$ in. wide rounded at each end. Holes $\frac{1}{8}$ in. in diameter are bored at $\frac{1}{2}$ in. intervals from the far end and threaded to take a brass pin (23) $1\frac{1}{2}$ in. long which extends $\frac{3}{4}$ in. above the iron strip and is held in position by means of a screw washer above and a small nut below. The best working position is when the pin is in the fifth hole from the end.

A series of reducing gears from the motor finish up with a crank (24) giving a throw of $6\frac{1}{2}$ in. The connexion between the crank and the iron strip is made by an arm (25) 9 in. long with a socket at one end with four holes at $\frac{1}{2}$ in. intervals at the other. One of these holes fits over the pin in the iron strip and the socket slips over the pin of the crank.

For most purposes 100–120 revolutions per minute are found to be satisfactory with the stirrer moving backwards and forwards through an angle of 50° . But these details can be altered in accordance with the motor power available and the type of soil being examined.

(e) *The air pump and (e_1) manometer* (Pl. XXXIX, fig. 1, *e* and e_1 , Text-fig. 1, *e* and e_1 and Text-fig. 2 (a), *e*).

A Marco air pump is used. A pressure of 14–18 cm. of mercury is required to drive the air through the cotton-wool.

(f) *Electric motor* (Pl. XXXIX, fig. 1, *f*, Text-fig. 1, *f* and Text-fig. 2 (a), *f*).

A motor which develops $1/40$ H.P. is used, but a slightly more powerful one would be better.

(g) *Soil sedimentation tank* (Pl. XXXIX, fig. 1, *g* and Text-fig. 1, *g*).

This is made of galvanized iron. It is 17 in. high, 14 in. long and 6 in. wide. Two baffle plates (26) 9 in. deep are fixed centrally across the length of the tank, $1\frac{1}{2}$ in. apart. The chute (27) leading from the conical head rests on the surface of the liquid in the tank and the baffle plates serve to guide the insects and debris across the tank to the lip (28), which extends over the Buchner funnel.

On the front of the tank $1\frac{1}{2}$ in. from the top a narrow pipe (29) is fixed as an overflow to allow the surplus liquid to escape. The top of the lip is fixed at a level just above that of the outlet. The discharge outlet is in the form of a honey gate (30). The tank is provided with two handles (31) one in the front and one at the back.

(h) *The glass reservoir* (Pl. XXXIX, fig. 1, *h*, Text-fig. 1, *h* and Text-fig. 2 (a), *h*).

This is an ordinary aspirator bottle with a capacity of 10 litres. The outlet is filled with a rubber stopper through the centre of which passes a glass tube bent at right angles. The lower end of the tube is inserted in a rubber stopper in the top of a small tap funnel (32) which provides a visible flow for the liquid.

- (i) *Buchner funnel* and (j) *filter flask* (Pl. XXXIX, fig. 1, i and j and Text-fig. 1, i and j).

The funnel is $7\frac{1}{2}$ in. in diameter and the filter flask has a capacity of 2.24 litres.

The whole apparatus is mounted in a rectangular frame 3 ft. 6 in. \times 2 ft. 9 in. \times 12 in. wide, with the stirring mechanism and reservoir above. The brass tube (18) of the stirring mechanism passes through a hole in the top of the frame, $1\frac{1}{4}$ in. in diameter, which is then closed by two semicircular pieces of hard wood flanged to prevent them falling through the hole. These act as the upper bearing of the stirrer.

(4) *Technique*

Mr K. D. Baweja who is using the apparatus for his investigations on soil insects finds that the following procedure gives the best results.

A soil sample $3 \times 4 \times 9$ in. is taken with the help of iron plates designed for the purpose, and put in a bag which is closed and brought to the laboratory. It is first of all examined rapidly in an enamelled basin and all the macrofauna visible to the naked eye removed. As the soil of the Rothamsted Experimental Station is very flinty, some big stones which are likely to interfere with the subsequent treatment are separated and stirred in a vessel containing magnesium sulphate solution which is transferred to the machine during the course of the operation.

The soil after preliminary examination is poured on to the bottom sieve of the stirrer resting in the cylinder, the sieve being fixed some 4 in. above the brass box. The top sieve is then let down to its proper position and the cylinder closed with the conical head. It is now transferred to the wooden frame, connected with the air pump, and the chute of the conical head directed into the sedimentation tank so that the end of the chute is just touching the surface of the liquid. Sufficient quantity of the solution, including that from the large stones mentioned above is poured into the cylinder so as to bring its level just below the rectangular opening of the conical head. The air pump is then started and continued for 3–5 min. This helps to extricate the animals not embedded in the soil and cause them to float at once. The stirrer is now started by switching on the electric current. Uniform churning of the soil is maintained from 5 to 10 min. continuously or at repeated intervals of 3–4 min. In the process of stirring, debris along with the soil fauna starts rising up. If all the debris floats at once there is no need to work the stirrer again, but if it is found to take some time, as it frequently does, stirring is repeated once or twice as necessary. Soon after starting stirring, the

solution is let into the cylinder from the reservoir and its flow regulated to about 450 c.c. per min. As the level of the solution rises all the floating material passes into the sedimentation tank. The latter is provided with a small pipe $1\frac{1}{2}$ in. below the top edge connected with rubber tubing on the front side to drain off the excess of liquid. When no more debris is seen to rise, the air pump is stopped and the pipe closed. With the continuous discharge of the solution from the cylinder the level of the solution in the sedimentation tank soon begins to rise and the liquid starts floating down into the Buchner funnel. This is fitted with a white filter paper with a black one above. As the muddy solution from the cylinder passes over the sedimentation tank, the mud settles down and by the time the liquid reaches the funnel it has become nearly clear. Froth, grass blades, etc., stick to the sides of the conical head of the cylinder and the sedimentation tank and require brushing or sweeping with a rubber spatula. If the whole process is manipulated carefully very little solution is received in the funnel and this is conveniently filtered in a short time with the help of the suction pump. The residue left in the filter paper is washed rapidly with a small quantity of distilled or tap water and examined with a magnifying glass to remove the bigger organisms. The filter paper is then transferred to a glass dish and examined under a binocular microscope in strips for the more minute fauna. The whole process from taking the soil sample to the examination of residue takes from 30 to 40 min.

If churning is carried out too long, the more delicate organisms, such as aphides and thrips, are damaged; the amount of stirring is therefore according to experience.

The solution in the cylinder is drained out from the discharge outlet at the bottom and poured over a sieve (1.5 mm. mesh) into a bucket as a check to ensure the capture of larger worms that might have been missed in the preliminary examination or failed to float. But out of the observations made so far with the machine not a single specimen has thus been found.

The weight of the sample taken depends upon the moisture content of the soil. If the weather is dry it weighs from $5\frac{1}{2}$ to $6\frac{1}{2}$ lb., but if it is wet the weight increases to 7 and occasionally $7\frac{3}{4}$ lb. The apparatus has been dealing efficiently with the whole amount of the samples in regulated one treatment.

The total volume of liquid required for the apparatus is 47 litres:

Reservoir	10 litres
Cylinder and head	15 "
Tank	22 "

The solution may be used over and over again after standing to allow the soil to deposit. The loss is about 5 per cent per operation. Before using the solution again it must be tested with a hydrometer and if necessary brought up to strength by the addition of more magnesium sulphate.

III. RESULTS AND COMPARISON WITH OTHER METHODS

Preliminary experiments made by the writer showed that the number of insects and other animals obtained from soil with the apparatus was always much higher than that found by Morris's method.

Table I

*Bubbling and mechanical stirring as compared with hand stirring—
both in $MgSO_4$ solution*

Series No.	Type of soil examined	Numbers of soil animals								
		Ladell's machine			Stirring by hand			Difference		
		In-sects (a)	Other inverte-brates (b)	Total (c)	In-sects (d)	Other inverte-brates (e)	Total (f)	In-sects (a-d)	Other inverte-brates (b-e)	Total (c-f)
1	Allotment fallow	104	19	123	48	7	55	56	12	68
2	Grassy plot	96	27	123	92	6	98	4	21	25
3	Grassy plot	63	17	80	52	11	63	11	6	17

Dimension of soil sample = $3 \times 4 \times 9$ in.

Table II

Accuracy of determination on duplicate samples

Series No.	Type of soil examined	Numbers of soil animals						Ratio of	
		1st half soil sample			2nd half soil sample				
		(a)			(b)			(a)	(b)
		Insects	Other invertebrates	Total	Insects	Other invertebrates	Total		
1	Allotment (grass 2 years)	111	29	140	120	31	151	48.1	51.9
2	Allotment fallow	40	13	53	43	12	55	49.1	50.9

Dimension of soil sample = $3 \times 4 \times 9$ in.

Since October 1935 the machine has been used by Mr K. D. Baweja and before adopting it for routine work he submitted it to some tests which are summarized below. Table I shows the importance of the air bubbles in separating the fauna from the soil particles. In three trials with bubbles, 123, 123 and 80 individuals were found; without bubbles, 55, 98 and 63. Table II shows the accuracy of the determinations on duplicate samples. Each soil sample was thoroughly mixed and divided

Table III. Soil populations as determined by various methods

Series No.	Method	Year	Locality	Type of soil examined	No. of sample examined	Percentage of population			Notes
						Average invertebrates per acre in millions	Insects	Other invertebrates	
1	M'Allee	1907	Washington	(a) Forest floor	1	1.2	24.1	75.9	—
2	Cameron	1913-14	Cheshire	(b) Meadow land	1	13.6	80.2	19.8	About 73 % were ants
3	Morris	1916-17	Cheshire	(c) Glover's meadow	11	0.8	—	—	Insects only
4	Morris	1920-21	Cheshire	(d) Alluvial pasture	14	1.5	—	—	—
5	Thompson	1920-21	Rothamsted Exp. Sta.	(a) Lane field pasture	29	3.5	—	—	Insects only
6	Edwards	1925-6	Aberystwyth	(b) Broadbalk manured, dung	23	15.1	51.2	48.8	—
7	Ladell	1935-6	Rothamsted Exp. Sta.*	(c) Broadbalk unmanured	23	5.0	49.9	50.1	—
				(d) Pasture land	20	19.5	43.8	56.2	—
				Allotments:	20	27.2-44.6	48.3-69.2	51.7-32.8	—
				(a) Fallow	10	60.6	79.2	20.8	—
				(b) Grassy, 2 years	3	121.4	79.1	20.9	—
				(c) Broadbalk manured, dung (Feb. 1936, warm and moist)	1	106.1	80.8	19.2	—
				(d) Manured, dung (Feb. 1936, ground frozen)	1	40.8	80.8	19.2	—
				(e) Broadbalk unmanured (Feb. 1936, ground frozen)	1	25.6	85.7	14.3	—

* These figures were obtained by Mr K. D. Baweja working at Rothamsted in 1935-6.

Table IV. Insect populations as found by the present method in comparison with that of Morris

Series No.	Method	Year	Locality	Type of soil examined	No. of individuals per acre in millions										Notes
					Collembola	Thysanura	Orthoptera	Thysanoptera	Hemiptera	Lepidoptera	Coleoptera	Diptera	Hymenoptera	Pscophora	
1	Morris	1920-1	Rothamsted Exp. Sta.	Broadbalk manured, dung	2.39	0.11	0.01	0.02	0.01	0.03	0.79	1.41	2.96	—	Average of 23 observations
2	"	"	"	Broadbalk unmanured	0.69	0.04	0.01	0.02	0.00	0.04	0.37	0.54	0.71	—	Average of 23 observations
3	Ladell	1935-6	"	Allotments (a) Fallow	21.11	0.1	0.2	—	13.71	—	1.78	5.60	0.16	0.01	Average of 10 observations
4	"	"	"	(b) Grass	91.79	—	—	0.89	0.52	—	1.41	0.5	0.16	—	Average of 3 observations
5	"	1936	"	Broadbalk manured	58.55	18.29	—	—	—	—	3.11	3.66	—	—	One observation only
6	"	"	"	Broadbalk manured	27.18	0.52	—	—	—	—	1.04	3.14	—	—	Weather warm and moist. February
7	"	"	"	Broadbalk unmanured	19.66	—	—	—	—	—	1.57	0.63	—	—	Soil frozen. February
															Soil frozen. February

Series 3-7 were obtained by Mr K. D. Baweja working at Rothamsted in 1935-6. They are the first of a series of samples to be investigated throughout the year.

into two portions which were treated separately in the machine. The agreement between the two halves is remarkable. In one, 48.1 per cent of the fauna was found in one half and 51.9 per cent in the other; in the second sample, 49.1 per cent was found in one half and 50.9 per cent in the other. Table III shows the total animal population as determined by various methods on different types of soil. The figures are not fully comparable but they do show that by the use of the new apparatus very high figures are obtained. Table IV details the insect populations found by the present method, in comparison with Morris's figures obtained with Rothamsted soils. Morris found 7.73 million insects per acre on the dunged plots on Broadbalk. Mr Baweja's two observations are 85.7 million on a warm moist day in February and 32.9 million on a day in the same month when the soil was frozen hard. These observations on Broadbalk will be continued throughout the year in order to obtain a fuller and more accurate comparison. The average of ten observations (from October to February) on the fallow allotments gives a figure of 47.9 million insects and on the plots that had been grass covered for 2 years 95.6 million insects per acre.

These figures are sufficient to indicate the high efficiency of the apparatus described in this paper.

IV. SUMMARY

1. The published methods for the separation of insects and other animals from the soil are briefly described.

2. None of these methods being sufficiently rapid or efficient for soil fumigation investigations, a new method has been devised.

3. The principle of the method is flotation by a dense liquid (a solution of magnesium sulphate sp. gr. 1.11) aided by stirring of the soil and a stream of fine air bubbles passing from the bottom upwards through the mixture of soil and solution. This produces a froth which contains all the animals, and by raising the level of the liquid in the cylinder the froth is swept over into a tank filled with magnesium sulphate solution; here is deposited any soil that has been carried over. The clear solution is then passed on to a Buchner funnel where the insects and other animals are retained. A black filter paper is used in order to show up Collembola and other colourless organisms.

4. The apparatus has many advantages:

- (a) *Rapidity*. The separation taking 30–40 min. from taking the soil sample of 6–7 lb. weight.

(b) *Cleanliness*. The operation can be carried out in an ordinary laboratory.

(c) *Efficiency*. All the organisms are concentrated in a small volume of residue, less than 1 per cent of the original soil, and are undamaged.

(d) *Non-toxicity*. The great majority of the insects come through alive, so that eggs and larvae may be bred out for purposes of identification.

5. A full account is given of the technique employed. The solution may be used again and again as the clay settles very quickly and the clear liquid may be syphoned or poured off.

6. Very high figures have been obtained for the soil population. These are much in excess of those recorded by most other workers with agricultural soils; for example, from fallow land 60.6 million animals per acre were recorded and from new grass land, 121.4 million per acre.¹

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¹ Ford (7) found a population of 276.6 million per acre (both surface and soil animals) in a meadow near Oxford "covered with a thick mat of vegetation and rotting grass".

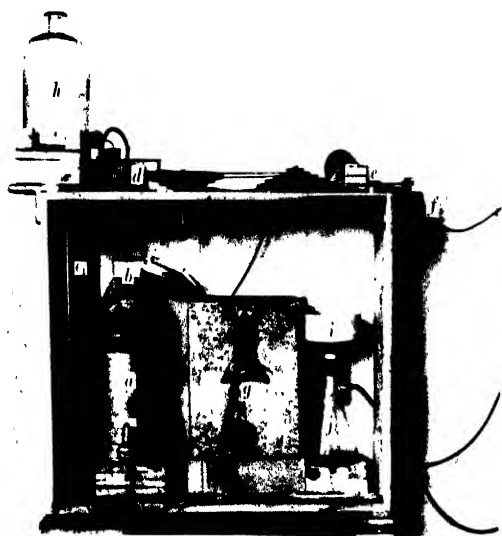


Fig. 1

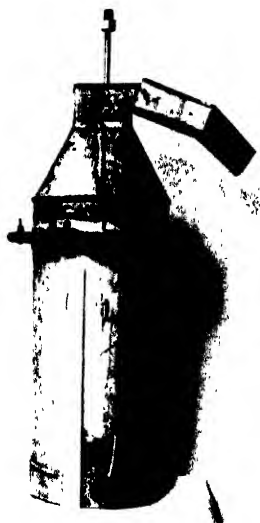


Fig. 2



Fig. 3

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EXPLANATION OF PLATE XXXIX

- Fig. 1. General view of the apparatus. *a*, mixing cylinder; *b*, conical head; *d*, stirring mechanism; *e*, air pump; *e*₁, manometer; *f*, electric motor; *g*, soil sedimentation tank; *h*, glass reservoir for solution; *i*, Buchner funnel; *j*, filter flask.
- Fig. 2. Improved cylinder with conical head. The watertight joint being made by two flanges with rubber washers in between. The central tube of the stirrer and air bubbler is seen above the rectangular opening of the head of the cylinder.
- Fig. 3. The stirrer and air bubbler. Showing the sieves and brass box with air tubes and stirring baffles. The two spanners are for use on the hexagonal nuts, one on the central tube and the other on the tube of the stirring mechanism.

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THE PROBLEM OF THE EVALUATION OF ROTENONE-CONTAINING PLANTS

II. *DERRIS ELLIPTICA*, *DERRIS MALACCENSIS* AND THE "SUMATRA-TYPE" ROOTS

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(With 2 Text-figures)

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INTRODUCTION

IN previous work reported in the first paper of this series (10) an examination was made of the chemical and insecticidal properties of two species of derris root at present in extensive use as insecticides, namely *Derris elliptica* and *D. malaccensis*. In this investigation, certain chemical factors were determined, and these in turn were then correlated with the insecticidal potencies of the roots to *Aphis rumicis*.

Of the limited number of samples examined, those of *Derris elliptica* were found to have a higher proportion of rotenone to total ether extract than was the case with the specimens of *D. malaccensis*, and Georgi & Teik in Malaya, working with roots of similar origin and kind, have also referred to this fact(3). From the analysis of twelve samples of derris from the Dutch East Indies, Spoon(7) found that those of *D. malaccensis* gave high ether extract but low rotenone, while the *D. elliptica* roots, showing less ether extract, were relatively richer in rotenone. It does not, however, seem possible to distinguish with certainty between roots of *D. malaccensis* and *D. elliptica*, by a consideration only of the relative proportions of rotenone to ether extract, since exceptional roots have been recorded. Thus Koolhaas(6) reports that a sample of *D. malaccensis* was found to contain 4.3–4.6 per cent of rotenone, amounting to 31–35 per cent of the total ether extract of the root, figures that may be thought to be more indicative of *D. elliptica*. More work is needed upon the relative proportions of rotenone to ether extract in authentic specimens of the two species before a general rule can be established.

A variety of derris is now in use, which although toxic, yields no rotenone by the normal method of separation of the rotenone-carbon tetrachloride complex. To such a root the name "Sumatra-type" has been given by Cahn & Boam(1) who show that rotenone is present in a "hidden" form, and may be induced to separate by the addition of an excess of the pure compound. It is a matter of some importance to characterize this type of root with greater precision in order to determine whether it approximates in chemical and physical properties to the more commonly known varieties of *D. elliptica* or *D. malaccensis*, or whether it is likely to prove to be a new species. In addition it is desirable that a suitable method of chemical evaluation, applicable to all types of derris, should be available in order to ascertain the degree of variation in the insecticidal efficiency of these roots due to cultivation or to varying climatic conditions.

We have shown (*loc. cit.*), in comparisons of *D. elliptica* with *D. malaccensis*, that neither the rotenone nor the ether extract could be relied upon to give a correct measure of the relative insecticidal activities of the roots, but that the determination of the mixed dehydro compounds, or of rotenone plus the dehydro compounds determined on the residual rotenone-free resins, gave a better assessment. In the present work we have compared, both chemically and biologically, a "Sumatra-type" root with samples of *D. elliptica* and *D. malaccensis* in order to ascertain to what extent the determination of the dehydro compounds, or of other

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chemical factors, will correctly evaluate the toxicity of the "Sumatra-type" root relative to the other two species examined.

EXPERIMENTAL

The "Sumatra-type" root (our No. W 170) was one kindly sent to us by the Cooper Technical Bureau, while the samples of *D. elliptica* and *D. malaccensis* used were two samples remaining from the tests carried out during the previous year, and reported upon in the first part of the present series. They were *D. elliptica* var. Sarawak creeping (our No. W 149) and *D. malaccensis* var. Sarawak erect (our No. W 151). The samples were all in the finely ground condition. The "Sumatra-type" root showed a crude rotenone content, determined by the "hidden" rotenone technique of Cahn & Boam (*loc. cit.*) of 1.95 per cent, this figure being reduced to 0.54 per cent of purified rotenone on trituration of the complex with alcohol saturated with rotenone. The *D. elliptica*

Table I

*The rotenone contents of "Sumatra-type" root (W 170),
D. malaccensis (W 151) and D. elliptica (W 149)*

Sample	Date of analysis		% of fresh root	
			Rotenone (crude)	Rotenone (purified)
"Sumatra-type" W 170	Oct. 1935	Ether, CCl ₄ , Cahn & Boam's "hidden" rotenone	2.01	—
	Feb. 1936	Ether, CCl ₄ , Cahn & Boam's "hidden" rotenone, recrystallization from CCl ₄	2.07	0.47
	Mar. 1936	Ether, CCl ₄ , Cahn & Boam's "hidden" rotenone, Cahn & Boam's trituration	1.95	0.54
<i>D. malaccensis</i> Sarawak erect W 151	Apr. 1933	Ether, CCl ₄ , recrystallization from alcohol	2.54	1.83
	Oct. 1935	Ether, CCl ₄ , Cahn & Boam's "hidden" rotenone, recrystallization from alcohol	2.70	1.81
	Feb. 1936	Ether, CCl ₄ , recrystallization from CCl ₄	3.13	1.87
	Mar. 1936	Ether, CCl ₄ , Cahn & Boam's trituration	2.84	1.93
<i>D. elliptica</i> Sarawak creeping (W 149)	Apr. 1933	Ether, CCl ₄ , recrystallization from alcohol	5.08	3.83
	Oct. 1935	Ether, CCl ₄ , Cahn & Boam's "hidden" rotenone, recrystallization from alcohol	5.07	4.05
	Feb. 1936	Ether, CCl ₄ , recrystallization from CCl ₄	5.26	4.07

In the recrystallization of the complex, allowance was made in each case for the solubility of rotenone in the solvent used.

gave figures of 5.08 per cent of crude, and 3.83 per cent of recrystallized rotenone, while the *D. malaccensis* figures were 2.54 and 1.83 per cent (Table I). It is of interest to note that there was no significant falling off in the rotenone contents of samples W 149 and W 151 on keeping the finely ground powders from April 1933 until March 1936 in tins at room temperatures in the region of 20°C. (see Table I).

INSECTICIDE TESTS

Biological trials using the three samples were carried out during the summer of 1935. Trouble was experienced in the early part of the summer by the incidence of disease in the stock of insects. Later, however, this was satisfactorily eliminated, but the number of biological trials it was possible to carry out was considerably reduced. The "Sumatra-type" root was compared alternately with the *D. elliptica* root W 149 and with the *D. malaccensis* root W 151. A weighed portion of each root was extracted with ether, the dried resin dissolved in alcohol, and a series of dilutions with 0.5 per cent saponin solution prepared. The alcohol content of each solution tested was adjusted to 5 per cent by volume, and the apparatus used was that previously described⁽⁹⁾. Adult apterous females of *Aphis rumicis* were used as test subjects, and comparisons only made between tests carried out on the same day. As in the previous work (*loc. cit.*), we have taken the percentages of badly affected, moribund and dead insects (B, M, and D per cent) observed on the second day's examination as indicating the toxic effects. The concentrations employed, expressed as mg. of root per litre, with the observed mortalities, are given in Table II and are plotted against the probit values in Figs. 1 and 2. The comparison of the "Sumatra-type" root with *D. elliptica* W 149 was made on 31 July, and the comparison with the *D. malaccensis* W 151 root on 12 August 1935. In the comparison of the "Sumatra-type" root with W 149, the former gave 50 per cent of badly paralysed and dead insects at a concentration equivalent to 685 mg., and the *D. elliptica* W 149 at a concentration equivalent to 238 mg. root per litre of spray fluid. In the later comparison of the "Sumatra-type" root with *D. malaccensis*, the concentrations giving 50 per cent "mortality" were equivalent to 763 and 338 mg. of root per litre respectively. The test insects used thus show a greater resistance in the later than in the earlier trials. In Table II, columns 2-8, are given the concentrations tested expressed in terms of various chemical determinations, to which detailed reference will be made later (pp. 886-893).

Table II

Comparison of the toxicities of the "Sumatra-type" root W 170, with D. elliptica W 149 and D. malaccensis W 151. Insect used Aphis rumicis. Fivefold replication, ten insects at a time. Results 2 days after spraying

Sample	Concentrations (mg./1000 c.c.) tested, in terms of										Toxicity results		
	Purified rotenone	Rotenone + dehydro compounds			Total dehydro compounds	"Toxic" resin ("rotenone equiv.")			Rotenone + "deguelin concn- trate"	No. of insects	Insects paralysed B+M+D %	S.E. % \pm	Probits of B+M+D
		on resin	soluble in ether after potash extraction	Resin soluble in ether after potash extraction		soluble in ether after potash extraction	soluble in ether after potash extraction	potash extraction					
"Sumatra-type" root W 170	Root 1250	6.8	20	49	102	39	31	39	38	50	96	4.0	6.751
	1000	5.4	16	39	82	31	23	31	31	50	86	6.0	6.080
	750	4.1	12	30	61	30	23	23	23	50	68	8.1	5.413
	625	3.4	10	25	51	25	19	19	19	49	36.7	6.6	4.660
	500	2.7	8	20	41	20	16	16	16	49	16.3	5.2	4.018
<i>D. elliptica</i> W 149	353	13.5	37	53	38	53	53	53	38	50	92	5.8	6.405
	282	10.8	30	42	30	42	42	42	31	40	55	6.6	5.126
	212	8.1	23	32	22	32	32	32	23	50	42	5.8	4.798
	176	6.8	19	26	19	26	26	26	19	50	18	2.0	4.065
	—	—	—	—	—	—	—	—	—	50	0	—	—
Control (saponin and alcohol solution)													
<i>D. malaccensis</i> W 151	547	10.0	36	49	43	45	36	45	43	50	91.3	3.7	6.360
	437	8.0	29	39	35	39	27	36	34	50	78.3	6.3	5.782
	328	6.0	26	29	26	29	23	27	26	49	33.5	6.5	4.574
	273	5.0	22	25	22	25	20	23	21	50	30.4	4.0	4.487
	219	4.0	17	20	17	20	18	17	17	49	17.9	6.6	4.081
"Sumatra-type" root W 170	1250	6.8	20	49	102	39	31	39	38	50	95.7	2.4	6.717
	1000	5.4	16	39	82	31	23	31	31	50	69.6	3.7	5.513
	750	4.1	12	30	61	30	23	23	23	49	40.1	8.4	4.749
	625	3.4	10	25	51	25	19	19	19	50	34.8	10.9	4.609
	500	2.7	8	20	41	20	16	16	16	50	15.2	3.7	3.972
Control (saponin and alcohol solution)										50	8	3.7	—

The B + M + D percentages are given allowing for control figure of 8 %. Standard errors calculated on percentages before allowing for control.

Comparison of toxicities of "Sumatra-type" root W 170 and *D. elliptica* W 149

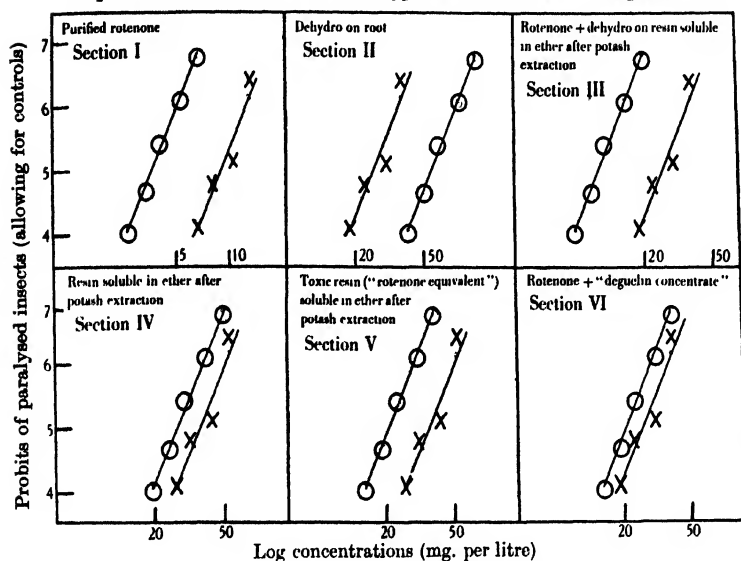


Fig. 1. Log concentrations in terms of different chemical values plotted against probits of badly paralysed insects (B, M and D per cent). ○ "Sumatra-type" root W 170 × *D. elliptica* W 149

Comparison of toxicities of "Sumatra-type" root W 170 and *D. malaccensis* W 151

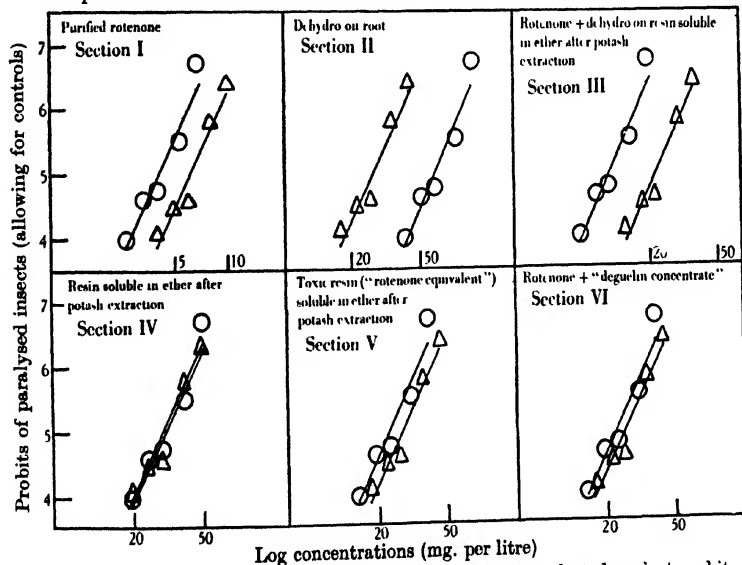


Fig. 2. Log concentrations in terms of different chemical values plotted against probits of badly paralysed insects (B, M and D per cent). ○ "Sumatra-type" root W 170, Δ *D. malaccensis* W 151.

CHEMICAL EXAMINATION OF THE ROOTS

Rotenone. In the determinations of the rotenone content of the "Sumatra-type" root the "hidden" rotenone method outlined by Cahn & Boam (*loc. cit.*) has been used, following failure to induce crystallization on dissolving in carbon tetrachloride the resin extracted from the root by ether. In the trituration of the product it was found preferable to filter at 0°C., using alcohol saturated with rotenone at this temperature. The purification of the complex resulted in a much reduced figure, the crude product appearing to be contaminated by resin. Reference to Table II and Figs. 1 and 2 shows that the estimation of purified rotenone does not give a correct assessment of the relative insecticidal efficiencies.

Ether extract. The determination of the ether extract was made by extracting 5 g. of root with anhydrous ether, the resin being dried to constant weight in an electric oven kept at 100°C. On the resin so obtained, the percentage of methoxyl content was determined by the method of Clark(2), and from it that of the root calculated. The ether extract or methoxyl figures (Table III) clearly do not assess the activities of the roots.

Table III

*Analytical data for "Sumatra-type" root W 170,
D. malaccensis W 151 and D. elliptica W 149*

	% of fresh root		
	"Sumatra- type" W 170	<i>D. malaccensis</i> W 151	<i>D. elliptica</i> W 149
Moisture	6.70	6.48	6.42
Rotenone (crude)	1.95	2.54	5.08
Rotenone (purified)	0.54	1.83	3.83
Ether extract	18.33	18.56	17.50
Methoxyl (on ether extract)	2.48	2.64	2.60
Benzene extract	19.02	19.42	17.94
Methoxyl (on benzene extract)	2.51	2.70	2.61
Total dehydro compounds	8.16	7.90	10.75

Dehydro compounds. The dehydro compounds were determined on the resin extracted by ether from 25 g. of the "Sumatra-type" root by the modification of the method of Takei *et al.*(8) given in paragraph 2 on p. 587 of our previous communication (*loc. cit.*). The mixed dehydro product was equivalent to 8.16 per cent of the root, and melted at 201°C. This is of the same order as the yield of the mixed dehydro compounds obtained by the same method from the resin of *D. malaccensis* W 151 (7.90 per cent). From biological trials, however, the *D. malaccensis* was

seen to be approximately twice as toxic as the "Sumatra-type" root. Thus it is evident that in the comparison of these two roots the determination of the dehydro compounds is inadequate as a means of obtaining the relative activities, the "Sumatra-type" being overvalued by this means with respect to the *D. malaccensis*. The results of the analyses are given in Table III.

Treatment of the resins with dilute alkali, and the determination of dehydro compounds on the resulting fractions

It was decided at this stage to carry out a fractionation of the resins by means of dilute alkali, in order to determine to what extent the presence of toxicarol in the resin influenced the figure for the mixed dehydro compounds obtained. The use of dilute aqueous alkali in removing the toxicarol fraction of the resin has been suggested by Haller & La Forge⁽⁴⁾ and utilized by Jones *et al.*⁽⁵⁾ in their work on the chemical evaluation of derris. In the first fractionations carried out, portions of 50 g. each of the "Sumatra-type", *D. malaccensis* and *D. elliptica* roots were extracted with ether, and with the latter two the ether was removed and the residue taken up with carbon tetrachloride as in the normal rotenone determination. The rotenone separating was filtered, and recrystallized from alcohol, the alcoholic mother liquors being retained. From the carbon tetrachloride solutions the solvent was removed as completely as possible *in vacuo*, and the residues dissolved in ether. The ether solutions of the resins from each root were extracted three times in a separating funnel with 50 c.c. of 5 per cent aqueous potash solution. The alkaline extracts were combined, washed with ether, the ether layers separated and added to the main ether solution. After washing with water, the ether was distilled and the resins dissolved in absolute alcohol, the alcoholic filtrates from the earlier rotenone recrystallizations being incorporated at this point. The yields of dehydro compounds from the ether-soluble resins were then determined.

In the case of the "Sumatra-type" root, the alkali treatment of the ether solution of the resin resulted in the production of a copious yellow precipitate, and this also appeared in the alkaline extract of the *D. malaccensis* resin. The extract of the *D. elliptica* resin, however, showed no precipitate, the alkaline layer appearing brown-red in colour. The precipitates were filtered, washed, and taken up in ether after treatment with dilute hydrochloric acid. The clear brown solutions after removal of the yellow precipitates and the alkali extract of the *D. elliptica* resin were also acidified, and the freed resins taken up in ether. The solvent

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was removed in each case and the residue, after solution in alcohol, subjected to the dehydro process as outlined. The determinations on the potash-soluble fractions were unsatisfactory, mixed resinous products resulting. The yields of the dehydro compounds from the ether-soluble resins and from the resins recovered from the yellow precipitates, expressed as percentages of the fresh root, are given in Table IV. In addition, the percentage recoveries of dehydro compounds from the weights of resins actually submitted to the dehydro process are given in brackets.

Table IV
*Dehydro compounds determined on fractions of resins
following alkali treatment in ether solution*

	"Sumatra- type" W 170		<i>D. malaccensis</i> W 151		<i>D. elliptica</i> W 149	
	% of root	% of resin	% of root	% of resin	% of root	% of resin
Dehydro compounds on total ether extract	8.16	(44.5)	7.90	(42.6)	10.75	(61.4)
Recrystallized rotenone removed	—	—	1.64	—	4.36	—
Dehydro compounds on resin soluble in ether after potash extraction	1.56	(43.3)	5.01	(54.6)	6.12	(58.6)
Dehydro compounds on resin from yellow precipitate extracted by potash	3.58	(33.2)	1.73	(48.0)	—	—

In the case of the "Sumatra-type" root, the yield of the dehydro compounds determined on the resin soluble in ether after the potash treatment, amounted to 1.56 per cent of the root, and presumably resulted from the rotenone and deguelin present. By reference to the methoxyl figures given in Table V, the resin soluble in ether after potash treatment in the case of the "Sumatra-type" root was less rich in "toxic" material than were the corresponding resins from the other two roots, but even so the recovery of dehydro compounds from it (43 per cent) was unduly low.

With the *D. malaccensis* and the *D. elliptica*, the combined figures for the separated rotenone and the dehydro compounds determined on the ether-soluble resins after the removal of toxicarol, amounted to 6.65 and 10.48 per cent of the roots respectively. The method of determination of the dehydro derivatives of the active principles is not a quantitative one, particularly so in the case of the "toxicarol" resin, where a yield of somewhat less than 50 per cent is obtained. In addition, the unsuitability of the method as a means of assessing the activity of the "Sumatra-type" root *vis-à-vis* *D. malaccensis* and *D. elliptica* is clearly demonstrated by

Figs. 1 and 2 (sections 2 and 3). In section 2 of each figure, the equivalent log concentrations of the dehydro compounds determined on the total ether extracts are plotted against the probits of the badly paralysed insects. In each case, the lines for the *D. malaccensis* and *D. elliptica* roots lie to the left of that for the "Sumatra-type" root, indicating that the latter, relative to the other two, is less toxic than its dehydro figure of 8.16 per cent would lead us to expect, if we assume the dehydro figures provide a correct measure of activity. In this case, the dehydro figure clearly overestimates the activity of the "Sumatra-type" root. When, however, we take the values obtained for the separated rotenone plus the dehydro compounds determined on the residual toxicarol-free resins, there is a reversal in the positions of the lines, and now the activity of the "Sumatra-type" root is undervalued relative to the other two roots.

Fractionation of the resins in ether solution

This was carried out using as before 5 per cent aqueous potash solution, the amounts of "toxic" constituents in each fraction being assessed by means of methoxyl determinations carried out upon the products isolated. With each of the three types of root under consideration, two portions of 5 g. were extracted with anhydrous ether, the solvent removed from one of the duplicate tests, the resin dried to constant weight at 100°C., and the methoxyl content determined. The second ether solution (50 c.c.) in each case was extracted successively with 10, 5 and 5 c.c. of 5 per cent aqueous potash, the alkaline extract being washed as before with ether. The resins from the precipitates and alkali-soluble fractions were recovered by acidification with dilute hydrochloric acid and solution in ether. The ether solutions were washed, dried with anhydrous sodium sulphate, the solvent removed, and the resins dried at 100°C. until of constant weight (Table V). Methoxyl determinations were then made upon the fractions obtained, special care being taken to ensure that all traces of ether had been removed; small amounts of ether were found to be tenaciously retained, it being necessary to heat the resins *in vacuo* in order to remove final traces of solvent. From the methoxyl values of the fractions, the contents of active principles of each, based in the case of the ether-soluble resins upon the methoxyl content of rotenone and deguelin of 15.74 per cent, and in the case of the alkali-extracted resins upon the methoxyl content of toxicarol of 15.12 per cent, were determined. The figures for the "toxic" constituents of each fraction, expressed as percentages of the fresh root, are given in Table VI.

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Table V

*Fractionation of the resins, in ether solution,
by means of potash solution*

	"Sumatra-type" W 170	<i>D. malaccensis</i> W 151	<i>D. elliptica</i> W 149
Total ether extract	18.33 (13.53)	18.56 (14.13)	17.50 (14.87)
Resin soluble in ether after potash extraction	3.93 (12.41)	8.95 (14.48)	14.94 (15.70)
Resin extracted and precipi- tated by potash	6.27 (15.40)	4.16 (14.91)	—
Resin extracted and soluble in potash	7.19 (11.83)	4.50 (10.92)	2.30 (6.94)

Figures expressed as percentages of fresh root. Percentage of methoxyl in each fraction given in brackets.

Table VI

*"Toxic" constituents in each fraction based upon
the methoxyl contents*

	"Sumatra- type" W 170	<i>D. malaccensis</i> W 151	<i>D. elliptica</i> W 149
"Rotenone plus deguelin" (soluble in ether after potash extraction*)	3.10	8.23	14.90
"Toxicarol" equivalent (extracted and precipitated by potash†)	6.27	4.10	—
"Toxicarol" equivalent (extracted and soluble in potash†)	5.63	3.25	1.06
Total "toxic" constituents	15.00	15.58	15.96

Figures expressed as percentages of fresh root.

* Based on methoxyl content of rotenone and deguelin of 15.74 per cent.

† Based on methoxyl content of toxicarol of 15.12 per cent.

With the "Sumatra-type" and *D. malaccensis* roots 95 per cent, and with the *D. elliptica* 98.5 per cent of the total ether extract were recovered in the fractions separated. The combined "toxic" constituents for each root based upon the methoxyl contents of the resins separated were of the order of 82-90 per cent of the total ether extract figures.

We again see from the probit-concentration curves (Figs. 1 and 2) both for the ether-soluble resins after potash treatment (section 4), and also for the calculated "rotenone plus deguelin" contents of these fractions (section 5), that the "Sumatra-type" root is undervalued with respect to the other types. It would appear that the material extracted by alkali, or a related compound, is playing some part in the insecticidal action of the root.

The effect of the substitution of saturated baryta solution for the potash upon the amounts of resin remaining in solution in the ether was determined. The ether extract from 5 g. of each root was extracted three

times successively with 10 c.c. of baryta, the ether layers dried as before, and the amounts of resin in solution determined. The figures for the ether-soluble resin for the "Sumatra-type" root, *D. malaccensis* and *D. elliptica* (3.76, 9.12 and 14.80 per cent of the fresh roots respectively) were in close concordance with the amounts of resin remaining in the ether after the use of potash.

The fractionation of the resins with 5 per cent aqueous potash was repeated, using in this case benzene as solvent. Duplicate portions of 5 g. of each root were extracted with benzene, one test being used in each case for the determination of the percentage of extract. Fractionation of the second solution was carried out, using successively 10, 5 and 5 c.c. of aqueous potash solution. In no case was a precipitate formed, the alkali extracts appearing as brown solutions. The total alkaline extracts of each root were washed with benzene, which was added to the main benzene solution. The benzene extracts were dried, and the amounts of resin in solution determined. It was found that, after acidification and solution in ether, only a relatively small percentage of the resin was extracted by the potash from the benzene extracts of the three types of root treated. The results are given in Table VII.

Table VII

*Fractionation of the resins, in benzene solution,
by means of potash solution*

	"Sumatra- type" W 170	<i>D. malaccensis</i> W 151	<i>D. elliptica</i> W 149
Total benzene extract	19.02 (13.22)	19.42 (13.92)	17.94 (14.57)
Resin soluble in benzene after potash extraction	14.54 (14.23)	15.34 (14.58)	15.42 (15.61)
Resin extracted and soluble in potash	3.02	2.87	1.84

Figures expressed as percentages of fresh root.

Methoxyl content as percentage of each fraction given in brackets.

Further fractionation of the resins that had remained soluble in the benzene on extraction with potash was then carried out by dissolving them in 50 c.c. of ether and extracting successively with 10, 5 and 5 c.c. of saturated baryta solution. By this procedure the yellow precipitates, separating before in the case of the "Sumatra" and *D. malaccensis* roots on extracting with potash or baryta from ether solutions, were again obtained, with a corresponding reduction in the amounts of resin remaining in solution in the ether layers. The resins extracted by the alkali were, as before, recovered by solution in ether after acidification with

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dilute acid. The results are given in Table VIII. Methoxyl determinations on the fractions separated, where carried out, are given in brackets.

Table VIII

Fractionation of the resins soluble in benzene after potash extraction by means of baryta, using ether as solvent

	"Sumatra-type" W 170	<i>D. malaccensis</i> W 151	<i>D. elliptica</i> W 149
Resin soluble in benzene after potash extraction	14.54 (14.23)	15.34 (14.58)	15.42 (15.61)
Resin soluble in ether after baryta extraction	3.55 (12.48)	7.32 (14.84)	13.34 (15.50)
Resin extracted and precipitated by baryta	9.51 (14.83)	6.27 (14.55)	0.76
Resin extracted and soluble in baryta	0.57	0.38	0.41

Figures expressed as percentages of fresh root.

Methoxyl content as percentage of each fraction given in brackets.

The potash treatment of the benzene solutions of the resins has effected a partial purification, as judged by the methoxyl values, although some loss of methoxyl has occurred as well. The amounts of "toxic" resin, calculated from a methoxyl content of rotenone and deguelin of 15.74 per cent, remaining soluble in the ether after the two alkaline treatments, although slightly lower, are of the same order as the amounts given in Table VI. When the results given in Table VIII are compared with those in Table V it is seen that the baryta extraction has precipitated a larger proportion of the material extracted by alkali than has potash. In no case did the baryta-soluble material exceed 1 per cent of the root. The outstanding effect obtained in these tests was the inability of the potash to effect the separation of the potassium salt of toxicarol or of its precursor, from a benzene solution of the "Sumatra" or *D. malaccensis* resins.

Fractionation of the resins using petroleum ether as precipitant

It is known that rotenone, deguelin and toxicarol are almost insoluble in petroleum ether. This fact was utilized in an attempt to purify the resins by precipitation of the toxic constituents from concentrated ether solution by means of petroleum ether. Our experience, based upon a number of trials, was that this procedure did not effect a sufficiently quantitative separation of the active principles to justify its use for purposes of evaluation.

*Preparation of "deguelin concentrates" after
petroleum ether extraction of the roots*

Haller & La Forge (*loc. cit.*) have shown that although the solubility of the toxic constituents of derris in petroleum ether is low, prolonged extraction will separate a resin containing a high proportion of the active principles, contaminated with extraneous material relatively small in amount by comparison with extracts using other solvents. They state that such an extract is particularly suitable for the examination of the non-crystallizable constituents. We have used prolonged extraction with petroleum ether in a Soxhlet apparatus in a further endeavour to prepare, as quantitatively as possible, "deguelin concentrates" from the three types of root under investigation.

Portions of 20 g. of the "Sumatra", *D. malaccensis* and *D. elliptica* roots were extracted with petroleum ether, with rapid refluxing, for 55 hours, the root material being taken out and mixed at frequent intervals to ensure penetration of the solvent. The petroleum ether was removed, the extracts dissolved in a little ether, the solutions seeded, and placed in an ice-chest for 2 days. Some crystals separated, particularly in the *D. elliptica* extract and these were removed. The ether solutions were then extracted with dilute potash, washed with water, dried over sodium sulphate and after concentration to a small bulk, again placed in the ice-chest for 5 days. A further amount of rotenone was deposited from the *D. malaccensis* extract. The weights of the resins remaining in solution in the ether were then determined (Table IX).

Table IX

*Preparation of "deguelin concentrates" following
petroleum ether extraction of the roots*

	% of fresh root		
	"Sumatra- type" W 170	<i>D. malaccensis</i> W 151	<i>D. elliptica</i> W 149
Rotenone separating from ether solution	0.45	1.62	1.88
Resin ("deguelin concentrate") remaining in ether solution	2.65	6.19	8.97

The combined figures for the separated rotenone and resin remaining in solution in the ether were 3.10, 7.81 and 10.85 per cent of the roots examined. The activity of the "Sumatra-type" root is again somewhat undervalued by the figure of 3.10 per cent, both with respect to the

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D. malaccensis (7.81 per cent), and to the *D. elliptica* (10.85 per cent) (see Table II and Figs. 1 and 2, section 6).

NATURE OF THE RESIN RECOVERED FROM THE MATERIAL PRECIPITATED BY POTASH FROM AN ETHER EXTRACT OF THE "SUMATRA-TYPE" ROOT

On acidification of the yellow precipitate and extraction with ether a yellow resin resulted, which has not so far been induced to crystallize¹. The resin had a methoxyl content in close agreement with the theoretical value for inactive toxicarol (15.12 per cent), and was optically active, a solution in benzene showing a laevo-rotation. On addition of potash in methyl alcohol to such a solution, an immediate change to a dextro-rotation, followed by a decline in rotation, took place, and this reaction has been found to be characteristic of the "Sumatra-type" and *D. malaccensis* resins. It will be discussed in more detail in Part III of the present series (11). When the resin was dissolved in alcohol and refluxed with addition of potash, yellow crystals soon separated, and these, on recrystallization from acetic acid, melted at 224°C. Inactive toxicarol, obtained from "Sumatra-type" resin by the method of Cahn & Boam (*loc. cit.*) melted at 221°C. The insecticidal activity of the resin has not yet been determined, but further investigation of this interesting derivative is called for, as it appears to be rich in a precursor of the inactive toxicarol.

THE EFFECT OF THE REMOVAL OF TOXICAROL UPON THE SEPARATION OF ROTENONE FROM CARBON TETRACHLORIDE SOLUTIONS OF "SUMATRA-TYPE" AND *D. MALACCENSIS* RESINS

We have found, with a "Sumatra-type" root from which no rotenone separates by the normal procedure, that after the removal of the preponderating toxicarol by alkali treatment of the ether extract, the naturally occurring rotenone separates readily on taking up the residual resin in carbon tetrachloride. The product furthermore appears to be but little contaminated by resin. As opposed to this, quite considerable loss in apparent rotenone takes place on purification of the complex obtained by the Cahn & Boam "hidden" rotenone technique.

Duplicate portions of 4 g. each of the resin extracted by ether from the "Sumatra-type" root W 170, were taken. To one was added 10 c.c. of carbon tetrachloride, the resin dissolved, the solution cooled, seeded and kept in the ice-chest for 2 days. No crystals separated. One gram of pure rotenone was added and the determination of the "hidden" rotenone

¹ An optically active crystalline compound possessing insecticidal properties was isolated from the resin shortly after this paper was sent to press.

carried out by the method of Cahn & Boam (*loc. cit.*). The carbon tetrachloride mother liquor, on cooling and standing, deposited a further small amount of complex, and this was added to the main bulk of crystals. The crude complex, giving a figure of 1.95 per cent of "hidden" rotenone in the root, was purified by trituration, the purified rotenone being 0.54 per cent of the root. The second portion of resin, in 50 c.c. of ether solution, was extracted three times with 20 c.c. of 5 per cent potash, the alkaline extracts washed with ether, the combined ether solutions washed with water, dried over anhydrous sodium sulphate, the ether removed, and the residual resin taken up in 10 c.c. of warm carbon tetrachloride. After standing overnight, the first crystals were filtered, the mother liquor concentrated, and the final yield of complex obtained. The rotenone content calculated from the complex so obtained, was 0.47 per cent of the root. On purification by trituration this figure fell to 0.40 per cent. In the case of the complex separated by the "hidden" rotenone method, the product was dirty yellow in appearance, while the crystals separating after alkaline treatment were almost colourless.

The tests were repeated using a second "Sumatra-type" root (our No. W 180). The Cahn & Boam method was carried out as before on 4 g. of resin, while for the alkali treatment, followed by separation of the complex, the resin extracted by ether from 50 g. of root was used. In this instance, the complex was purified by recrystallization from alcohol, a correction being made for the rotenone retained by the solvent.

In previous rotenone determinations on the *D. malaccensis* W 151 root the crude complex had been highly coloured, and had shown a considerable loss in apparent rotenone on purification. The effect of alkali pretreatment of the resin of this root was therefore determined. In this case, the use of the "hidden" rotenone technique was not necessary, the complex, equivalent to 2.84 per cent of "crude" rotenone, separating overnight. On purification by alcoholic trituration the product lost much of its yellow colour, and a purified rotenone figure of 1.93 per cent of the root resulted. The "crude" rotenone determined on the duplicate portion of resin from which the toxicarol had been removed by extraction with alkali, was 1.96 per cent, the product being colourless. On purification by trituration, this figure was reduced to 1.70 per cent of rotenone in the root. The results are tabulated in Table X.

In the case of the "Sumatra-type" roots either the presence of large amounts of resinous material rich in toxicarol prevents the separation from carbon tetrachloride solution of the rotenone present, or potash treatment removes some other inhibitor of crystallization. The complex,

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which subsequently separates readily, is then obtained in an amount agreeing relatively closely with the figure obtained for the purified product by the normal method.

Table X

Separation of rotenone from carbon tetrachloride solutions of the resins with and without removal of toxicarol

	% of fresh root	
	Crude rotenone %	Purified rotenone M.P. (°C.) %
"Sumatra-type" W 170:		
Ether, carbon tetrachloride, Cahn & Boam's "hidden" rotenone method	1.95	0.54 (161)
Ether solution extracted with potash, carbon tetrachloride	0.47	0.40 (158)
"Sumatra-type" W 180:		
Ether, carbon tetrachloride, Cahn & Boam's "hidden" rotenone method	2.15	0.67 (161.5)
Ether solution extracted with potash, carbon tetrachloride	0.89	0.66 (159)
<i>D. malaccensis</i> W 151:		
Ether, carbon tetrachloride	2.84	1.93 (158.5)
Ether solution extracted with potash, carbon tetrachloride	1.96	1.70 (159)

More work is needed upon the suitability of alkali as a means of reducing the concentration of the inhibitor of rotenone crystallization. There is the possibility of the alkali causing a loss, by degradation, of the rotenone present, while oxidation products may be formed during the process of extraction. In this connexion, we have observed small, though varying amounts of a pale yellow material, melting in the region of 208°C., separating from the ether layer. It may well be, however, that such a pretreatment, suitably controlled, could form the basis of a standard method of rotenone determination applicable to the main types of derris root.

DISCUSSION

The work described was carried out in an endeavour to characterize more definitely on a chemical basis, the "Sumatra-type", *D. malaccensis* and *D. elliptica* roots, with a view to their evaluation by chemical means. The "Sumatra-type" root examined contained small amounts of rotenone and deguelin, while some 70 per cent of its resin was removed from an ether solution by treatment with potash. From the fraction precipitated by the alkali an optically active resin, appearing rich in toxicarol, was

obtained. The root was characterized by the inability of the rotenone to separate directly from a carbon tetrachloride solution of its resin, and this inhibition appeared to be due to the preponderance of the material extractable by potash.

The *D. malaccensis*, from its chemical properties, occupied a position intermediate between the "Sumatra-type" root and the *D. elliptica*, in that it contained greater amounts of both rotenone and deguelin, with a corresponding decrease in the content of alkali-extractable resin. With the *D. malaccensis*, this appeared to be insufficient in amount to inhibit the crystallization of the rotenone. The separation of rotenone is apparently dependent upon the relative proportion of rotenone to other resins in the carbon tetrachloride solution, and takes place when the proportion of rotenone to the inhibiting material is sufficiently high, a condition achieved either by the addition of the pure compound, as in Cahn's method, or by the removal of the non-crystallizable resin by treatment with alkali. Further work is needed, however, on this question before the factors influencing the separation of the complex are fully understood.

The *D. elliptica* differed markedly from both the "Sumatra-type" and *D. malaccensis* roots, in that very little material was extracted by potash from an ether solution of its resin, the extract showing no formation of precipitate. The rotenone present separated readily, and showed little contamination by resin. The "Sumatra-type" root is thus much more closely akin to the *D. malaccensis* than it is to the *D. elliptica* root.

Throughout the work, we have evidence that, although the insecticidal activity of the toxicarol as it occurs in the root is probably not comparable with that of rotenone, the preponderating amount present plays a definite part in the final toxic value of the "Sumatra-type" root. In all methods of attempted evaluation after the removal of the toxicarol the activity of the "Sumatra-type" root was undervalued, relative to the other roots, by the estimates obtained of the residual combined rotenone and deguelin. This undervaluation of the "Sumatra-type" root was greater in the comparison with the *D. elliptica* than it was in the tests with the *D. malaccensis*, a root of more similar type.

The dehydro method as a means of assessing the toxicity of the "Sumatra-type" root is inadequate and it would appear that the evaluation of derris types will only be possible when more is known of the nature and activities of the precursors in the root of the various crystalline and relatively inactive derivatives that have been isolated.

SUMMARY

1. The determinations of purified rotenone, ether extract, dehydro compounds, ether-soluble resin after potash treatment, and of the rotenone plus "deguelin concentrates" are each shown to be inadequate as a means of assessing the relative insecticidal activities of the "Sumatra-type", *D. malaccensis*, and *D. elliptica* roots.

2. The toxicarol present in the "Sumatra-type" derris appears to play a small but definite part in the insecticidal activity of the root.

3. The resin recovered from the material precipitated by potash from an ether extract of the "Sumatra-type" root is optically active, and appears to be rich in the precursor of inactive toxicarol.

4. Rotenone, if present, will separate readily from a carbon tetrachloride solution of "Sumatra-type" resin from which the toxicarol has been removed. The possibility of a standard method of rotenone determination, dependent upon suitable pretreatment of the resins, is suggested.

We wish to express our indebtedness to the Copper Technical Bureau for their kindness in supplying us with samples of the "Sumatra-type" root. We also wish to thank Dr S. Krishna of the Forestal Department of Northern India for valuable assistance.

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THE PROBLEM OF THE EVALUATION OF ROTENONE-CONTAINING PLANTS

III. A STUDY OF THE OPTICAL ACTIVITIES OF THE RESINS OF *D. ELLIPTICA*, *D. MALACCENSIS* AND THE "SUMATRA-TYPE" ROOTS

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(With 4 Text-figures)

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INTRODUCTION

ONE of the chief difficulties met with in attempting the assessment of the insecticidal value of the rotenone-containing plants, by either chemical or physical methods, lies in the great variety and complexity of types or strains. Not only is this true when different genera or species are considered, but also, within the same species marked contrasts have been noted. Henderson's(3) investigations bear witness to the wide botanical variation in habit of growth and leaf form of the *Derris* species. The chemical complexity of the extracts derived from these plants is no less confusing, and specimens of any one of the *Derris* species are known to vary widely in their chemical characteristics. Parts I(6) and II(5) of this series of papers deal with the more chemical aspects of the problem. We propose in this section to present a study of the optical activities

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of the extracts of three types of derris with the object of demonstrating as far as possible the extent and nature of this variation. Although only three samples are dealt with, it is hoped that the investigations described may prove a contribution to the differentiation of types or strains in this genus.

The importance of the study of optical rotations for this purpose is emphasized by the fact that the precursors of some of the isolated crystalline derivatives, e.g. deguelin and toxicarol, probably exist in the resins in an optically active state although the isolated crystalline products are devoid of this property. The optical rotation of rotenone in solution in various solvents has been investigated by Jones & Smith(4). It is the only constituent of a toxic nature the rotation of which is accurately known, and unquestionably it plays an important rôle in determining the optical activity of resins in which it occurs. The use of a rotation method for purposes of discrimination, evaluation and standardization is hardly warranted, without further information as to the parts played by other constituents of the derris resins. The "Sumatra-type" root presents certain possibilities in an investigation of this type, as it has been shown by Cahn & Boam(1) to contain large amounts of toxicarol. On the other hand, it is indicated in Part II of this series of papers that *D. elliptica* may be practically free from this constituent. *D. malaccensis* would seem to rank between these two in the chemical make-up of its resins. Inactive toxicarol is almost devoid of toxicity to insects and requires for its separation a treatment of the resin with alkali. A comparative study of the rotation of extracts of these three types of root and the changes induced by the addition of alkali, might make it possible to ascertain more exactly the relationship between them.

EXPERIMENTAL

The optical rotations of benzene solutions of rotenone and three different types of resin

Experiments were carried out to select solvents for extracting the resins and determining the rotations. Ether has the advantage of low-temperature extraction, but is difficult to use in the polarimeter. Thus it becomes necessary to take off the ether which these resins retain with some tenacity, and it was found that heating rotenone or derris resins at 100°C. slightly lowered their specific rotations. The rotations were therefore determined directly on the benzene extracts. This solvent

has, moreover, the advantage of giving higher rotations for rotenone than many others, and with the resins, solutions of which are often so dark coloured as to transmit very little light, makes possible the use of low concentrations. The benzene extract of the finely powdered root was made up to a known volume at 20°C. and the solid residue determined in an aliquot part. Dilutions of known concentrations at 20°C. were then prepared. The rotations were determined at 20°C. in a polarimeter, reading in circular degrees, using a 10 cm. tube fitted with a water jacket for controlling the temperature. An electrically heated sodium lamp provided the illumination. In Table I are given the data obtained for the three types of derris root examined and for rotenone; they are plotted in Fig. 1. Results obtained by Jones & Smith⁽⁴⁾ for rotenone in benzene solution are also plotted for comparison. The two sets of data are concordant, indicating that the rotenone used was of corresponding purity. Our sample was recrystallized several times from absolute alcohol and had a melting-point of 163–164°C. All the resins are laevo-rotatory, and the rotations when plotted against the concentrations are not strictly linear. The specific rotations similarly plotted fall approximately on parallel straight lines inclined to the axis of concentration (Fig. 1).

Table I

*Rotations of benzene solutions of extracts of derris root
of different types*

Name	Conc. of resin in g./100 c.c.	Conc. as g. of root/100 c.c.	Rotation α_D^{20}	Specific rotation $[\alpha]_D^{20}$
Sumatra type	17.802	100	- 9.82	- 55
Benzene extract	14.244	80	- 8.35	- 59
Extract = 17.802 %	8.545	48	- 5.45	- 64
	4.272	24	- 2.90	- 68
	2.136	12	- 1.50	- 70
<i>D. malaccensis</i>	19.058	100	- 14.17	- 74
Benzene extract	15.246	80	- 11.88	- 78
Extract = 19.058 %	9.148	48	- 7.65	- 84
	4.574	24	- 4.04	- 88
	2.287	12	- 2.08	- 91
<i>D. elliptica</i>	17.406	100	- 14.61	- 84
Benzene extract	13.925	80	- 12.14	- 87
Extract = 17.406 %	8.355	48	- 7.71	- 92
	4.177	24	- 4.01	- 96
	2.089	12	- 2.05	- 98
Rotenone	(1) 5.0	—	- 11.21	- 224
Benzene solution	1.0	—	- 2.32	- 231.5
	(2) 4.6730	—	- 10.49	- 224.5
	2.2655	—	- 5.20	- 229.5
	1.0635	—	- 2.46	- 231.0

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The question arises whether the rotatory power can be correlated with the insecticidal activity. The order of toxicity of these three samples of root judged at the median lethal dose for *Aphis rumicis* is "Sumatra-type" $1 < D. malaccensis$ $2.2 < D. elliptica$ 2.9 . When the rotations of the resins for the three samples are placed in order of magnitude at some equivalent concentration of the roots, e.g. 100 per cent, the samples are placed in the same order as that given by our toxicity data (Part II,

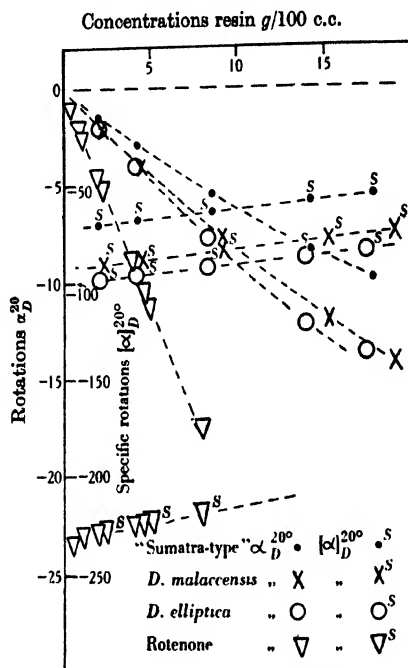


Fig. 1. Rotations and specific rotations of the benzene solutions of rotenone, and the resins of three types of derris root.

p. 884), assuming that the rotations are a measure of toxicity the order is $1 S < 1.4 M < 1.5 E$ (where S =Sumatra-type, M =*D. malaccensis* and E =*D. elliptica*). This quantitatively overvalues the "Sumatra-type" relatively to the other two. It should be noted, however, that the proportionality of the rotations changes with concentration.

Specific rotations in benzene of the ether extracts of the roots

Portions of 10 g. each of the "Sumatra-type", *D. malaccensis* and *D. elliptica* roots were extracted with ether, the solvent removed and the resins dried as completely as possible in an oven at 60°C . The resins, the weights of which varied between 1.74 and 1.91 g. were dissolved in

benzene, and the volumes adjusted to 25 c.c. at 20°C. The specific rotations of the "Sumatra-type", *D. malaccensis* and *D. elliptica* resins were -57.8° , -85.6° and -95.3° respectively. These figures increased to -65.5° , -90.8° and -100.7° on dilution of 10 c.c. of each solution to 25 c.c. with benzene, and redetermination of the rotations.

Further portions of each root were extracted with ether, and the solutions (about 50 c.c. in bulk) extracted successively with 20, 20, 10 and 10 c.c. of 5 per cent aqueous potash. The alkaline extracts were washed with ether, the ether solutions for each root combined, washed with water, dried over sodium sulphate, the solvent removed and the specific rotations of the ether-soluble resins determined in benzene solution. The weights of resin for the "Sumatra-type", *D. malaccensis* and *D. elliptica* roots were 0.410, 0.959 and 1.515 g., and the specific rotations determined at 20°C., were -72.6° , -112.6° and -94.5° respectively. The specific rotations, on dilution as before, were increased to -76.2° , -115.3° and 99.1° .

In the extraction of the "Sumatra-type" and *D. malaccensis* roots, the yellow precipitates formed in the alkaline layers were filtered and washed with water, and the resins, freed on acidification with dilute hydrochloric acid, taken up in ether. The ether solutions were washed free from acid, dried over sodium sulphate, and the solvent removed. The specific rotations of the resins were then determined in benzene solution. They were as follows: resin from the yellow precipitate from the "Sumatra-type", root -62.7° and for the resin from the *D. malaccensis* precipitate -67.5° . Haller & La Forge⁽²⁾ state that the precipitate formed on potash treatment of an ether solution of derris resin consists of the difficultly soluble potassium salt of toxicarol. On recovery of the potassium-free material from this by acidification and extraction with ether, the product, which so far we have not succeeded in obtaining in the crystalline condition¹, is shown to be optically active and laevorotatory in benzene.

We have, in addition, separated toxicarol from a "Sumatra-type" root by the method of Cahn & Boam (*loc. cit.*). The crystalline product melted at 221° and had a low solubility in ether and benzene. A 2 per cent solution in chloroform showed no optical rotation.

We have not been able to trace any simple correlation of the insecticidal powers of these roots and the rotations of either the crude resins or their constituent fractions. Much more information is needed on the rotations of the fractions of the derris resins and their relative toxicities.

¹ See, however, footnotes, pp. 894 and 915.

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The change of rotation on adding caustic potash in methyl alcohol to benzene extracts of derris root

Apart from rotenone, the several crystalline derivatives, e.g. deguelin and toxicarol isolated from derris root have been found optically inactive. There is some evidence to show that in the process of extraction these compounds have either been derived from some precursor or have undergone a process of racemization during extraction. As alkali was employed during the process it seemed advisable to ascertain what effect it had upon the rotation of the resins derived from the samples of derris root of the three types under examination. The benzene extracts used for the determination of the rotations at different concentrations (Table I) were employed. Aliquot parts (5 and 10 c.c.) were taken at 20° C., and known amounts of normal caustic potash solution in methyl alcohol were added; in addition, an equivalent volume of methyl alcohol was added to a further set in order to ascertain its effect, if any, upon the rotation. The higher concentrations of the resins became too dark under alkali treatment, and the investigations were therefore limited to the two lowest concentrations of the benzene extracts. The following distinguishing letters have been employed:

	Dilution	Resin g./100 c.c.
"Sumatra-type"	S.C.	4.272
	S.D.	2.136
<i>D. malaccensis</i>	M.C.	4.574
	M.D.	2.287
<i>D. elliptica</i>	E.C.	4.177
	E.D.	2.089

In addition, the effect was determined of the addition of methyl alcohol and the standard solution of caustic potash in methyl alcohol upon the rotations of different concentrations of rotenone and of the various fractions, isolated by caustic potash extraction of ether solutions of the resins, all in solution in benzene.

In Table II we have given all the salient data obtained for the "Sumatra-type". The equivalent of caustic potash to be added was calculated, assuming the resin to have the same molecular weight as rotenone. This assumption is not strictly valid, but it gave a rough approximation of the amount of caustic potash to be added and experience showed that within certain limits the amount of alkali added, although having a distinct bearing on the initial change of rotation, had only a slight one upon its subsequent rate of change.

In the table also the specific rotation of the mixture of methyl alcohol with the benzene solution of the resin is given, the resulting concentration of resin being calculated on the assumption that there was no change

in volume; the corresponding specific rotation of this concentration in benzene was determined from the graph in Fig. 1. It will be noted that the addition of methyl alcohol to the benzene solution of the "Sumatra-type" resin results in a reduction of the laevo-rotation. In a preliminary experiment on this resin, in which attempts were made to dissolve it in methyl alcohol, a portion of the resin was only slightly soluble in this

Table II

*Effect of addition of caustic potash in methyl alcohol
upon benzene extracts of "Sumatra-type" root*

Dilution S.D. 2.136 g. resin/100 c.c. = 12 g. root/100 c.c.

$\alpha_D^{20} = -1.50^\circ$. $[\alpha]_D^{20} = -70.2^\circ$.

2 c.c. methyl alcohol added to 5 c.c.

Conc. after addition of methyl alcohol = 1.526 g resin/100 c.c.

α_D^{20} after addition of methyl alcohol after

5 min. = -0.60° $[\alpha]_D^{20} = -45.2^\circ$

3 hours = -0.73° $[\alpha]_D^{20} = -47.8^\circ$

22 hours = -0.75° $[\alpha]_D^{20} = -49.2^\circ$

α_D^{20} for equivalent conc. in benzene = -1.07° $[\alpha]_D^{20} = -70^\circ$.

Dilution

S.C. 4.273 g. resin/100 c.c.

$\alpha_D^{20} = -2.9^\circ$. $[\alpha]_D^{20} =$

-67.9° .

4 c.c. methyl alcohol

added to 5 c.c.

Conc. after addition of

methyl alcohol =

2.3736 g./100 c.c.

α_D^{20} after 4 hours = -0.81° .

$[\alpha]_D^{20} = -34^\circ$.

α_D^{20} equiv. conc. in benzene

-1.65° . $[\alpha]_D^{20} = -6.95^\circ$.

Equivalents of potash. Resin calculated as having mol. wt. 394.

Time min.	1			2			3			1		
	$[\alpha]_D^{20}$	$[\alpha]_{k_1-330}$	k^*	$[\alpha]_D^{20}$	$[\alpha]_{k_1-205}$	k^*	$[\alpha]_D^{20}$	$[\alpha]_{k_1-115}$	k^*	$[\alpha]_D^{20}$	$[\alpha]_{k_1-195}$	k^*
1	+185	+116	—	—	—	—	+208	+131	—	—	—	—
2	—	—	—	+200.5	+116	—	—	—	—	+177	132	—
3	175	106	0.045	—	—	—	203	126	0.022	170	125	0.055
5	172	103	0.030	190	105.5	0.032	196	119	0.025	158	113	0.052
10	160	91	0.027	175	90.5	0.031	180	103	0.028	130	85	0.055
15	147	78	0.028	161	76.5	0.032	166	89	0.028	110	65	0.054
20	138	69	0.027	151	66.5	0.031	154	77	0.028	96	51	0.053
25	132	63	0.025	142	57.5	0.031	146	69	0.027	81	39	0.053
30	125	56	0.025	137	52.5	0.028	135	58	0.028	75	30	0.053
35	118.5	49.5	0.025	130	45.5	0.028	128	51	0.028	68	23	0.053
40	113	44	0.025	126	41.5	0.027	122	45	0.028	64	19	0.051
45	109	40	0.024	—	—	—	—	—	—	—	—	—
55	—	—	—	113	38.5	0.026	109	32	0.026	54	9	0.051
60	100.5	31.5	0.022	—	—	—	—	—	—	—	—	—
70	—	—	—	106	21.5	0.025	96	19	0.028	49	4	0.051
75	93	24	0.023	—	—	—	—	—	—	—	—	—
85	—	—	—	98	13.5	0.026	88	11	0.030	46	1	0.059
105	85	16	0.019	—	—	—	—	—	—	—	—	—
115	—	—	—	93	8.5	0.023	77	0	—	—	—	—
145	—	—	—	89	4.5	0.023	—	—	—	—	—	—
195	75	6	0.016	—	—	—	—	—	—	45	0	—
205	—	—	—	84	0	—	—	—	—	—	—	—
330	69	0	—	—	—	—	—	—	—	—	—	—
Mean $k = 0.026$				Mean $k = 0.0285$			Mean $k = 0.0275$			Mean $k = 0.053$		
Mean $k/c.c.$ methyl alcohol = 0.013				Mean $k/c.c.$ methyl alcohol = 0.014			Mean $k/c.c.$ methyl alcohol = 0.0135			Mean $k/c.c.$ methyl alcohol = 0.0133		

* Natural logs were used in calculating k .

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solvent and some of the fractions obtained when dissolved in methyl alcohol were dextro-rotatory in contrast with their solutions in benzene which were laevo-rotatory.

The addition of caustic potash in methyl alcohol causes a definite colour darkening of the solution and an instantaneous change from a laevo- to a dextro-rotation. The rotations then decline at first rapidly and afterwards more slowly. The readings were taken for the first and second minutes after mixing and afterwards every 5 min. until the change became so slow as to require a longer period for accuracy. Only one reading could be taken at these 5 min. intervals, so rapid was the early rate of change, but for the 15 min. intervals five readings, and for 30 min. and longer intervals ten readings were taken, the mean of each set being given in Table II. After a period the change became very slow and the solution darkened to such an extent as to render further readings impossible. In no instance did the dextro-rotation fall to zero with time, but approached a value which could be regarded as the end-point of the reaction. We are unable to state the nature of the product giving rise to this residual dextro-rotation or whether it was present in constant amount during the course of the decline in the rotatory power. It was considered preferable to take the final reading as the equilibrium point indicating a cessation of the main reaction. This value was therefore deducted from the other readings for purposes of calculating the velocity coefficient assuming the reaction to be unimolecular. The curves obtained by plotting the specific rotations against the time interval only approximate to the semi-logarithmic type, the value for the velocity coefficient $\frac{1}{t_2 - t_1} \log \frac{a - x_1}{a - x_2}$, in which, after deducting the final reading, a is the initial rotation and x_1 and x_2 the changes of rotation after time t_1 and t_2 , tends to decline with time, but, with many of the curves is approximately constant at their middle portion. For the lower concentrations of resin the mean velocity coefficient is practically independent of the amount of caustic potash added. The velocity coefficient for dilution S.C. is approximately double that found in the tests given by dilution S.D. Later work, the results of which are given below, demonstrated that the increase in velocity is not due to the increase in concentration of the resin, but to the amount of methyl alcohol added with the caustic potash. At the foot of Table II we have given the values for the ratio Mean $k/\text{MeOH c.c.}^1$. It is approximately the same in every case, namely 0.013-0.014.

¹ I.e. the number of c.c. of methyl alcohol added to 5 c.c. of the benzene solution of the resin.

The data for the similar treatment of *D. malaccensis* are given in Table III.

Table III

The effect of addition of caustic potash in methyl alcohol upon benzene extracts of D. malaccensis root

Dilution M.D. (2.287 g. resin/100 c.c. = 12 g. root/100 c.c.). $\alpha_D^{20} = -2.08^\circ$. $[\alpha]_D^{20} = -90^\circ$.				Dilution M.C. (4.574 g. resin/100 c.c. = 24 g. root/100 c.c.). $\alpha_D^{20} = -4.04^\circ$. $[\alpha]_D^{20} = -88.3^\circ$.					
2 c.c. methyl alcohol added to 5 c.c.		4 c.c. methyl alcohol added to 5 c.c.		4 c.c. methyl alcohol added to 5 c.c.		4 c.c. methyl alcohol added to 5 c.c.			
Conc. after adding methyl alcohol = 1.634 g./100 c.c.		Conc. after adding methyl alcohol = 1.27 g./100 c.c.		Conc. after adding methyl alcohol = 2.541 g./100 c.c.		Conc. after adding methyl alcohol = 2.541 g./100 c.c.			
$[\alpha]_D^{20}$ after adding methyl alcohol = -69° .		$[\alpha]_D^{20}$ after adding methyl alcohol = -61° .		$[\alpha]_D^{20}$ after adding methyl alcohol = -59.0° .		$[\alpha]_D^{20}$ after adding methyl alcohol = -59.0° .			
$[\alpha]_D^{20}$ for equiv. conc. in benzene = -91.5° .		$[\alpha]_D^{20}$ for equiv. conc. in benzene = -92° .		$[\alpha]_D^{20}$ for equiv. conc. in benzene = -91° .		$[\alpha]_D^{20}$ for equiv. conc. in benzene = -91° .			
Equivalents of potash added. Resin calculated as having mol. wt. = 394.									
Time min.	1			2			1		
	$[\alpha]_D^{20}$	$[\alpha]_{k_1}$ - $[\alpha]_{k_2=230}$	k	$[\alpha]_D^{20}$	$[\alpha]_{k_1}$ - $[\alpha]_{k_2=145}$	k	$[\alpha]_D^{20}$	$[\alpha]_{k_1}$ - $[\alpha]_{k_2=215}$	k
2	+111.4	72.8	—	119.6	88.9	—	107.8	97.2	—
5	109.6	71.0	0.006	103.9	73.2	0.065	94.1	83.5	0.051
10	102.2	63.6	0.017	85.0	54.3	0.062	73.6	63.0	0.054
15	94.3	55.7	0.021	71.6	40.9	0.060	60.6	50.0	0.051
20	86.9	48.3	0.023	63.0	32.3	0.057	48.4	37.8	0.052
25	81.4	42.8	0.023	55.9	25.2	0.055	40.2	29.6	0.052
30	75.6	37.0	0.024	—	—	—	33.5	22.9	0.052
35	71.0	32.4	0.025	—	—	—	29.1	18.5	0.050
40	—	—	—	43.3	12.6	0.052	—	—	—
50	60.6	22.0	0.025	—	—	—	20.3	9.7	0.048
55	—	—	—	37.0	6.3	0.050	—	—	—
65	53.2	14.6	0.026	—	—	—	15.7	5.1	0.047
80	50.2	11.6	0.024	—	—	—	—	—	—
85	—	—	—	33.0	2.3	0.044	—	—	—
95	—	—	—	—	—	—	13.0	2.4	0.040
110	44.7	6.1	0.023	—	—	—	—	—	—
145	—	—	—	30.7	0	—	—	—	—
155	—	—	—	—	—	—	11.0	0.4	0.036
170	40.4	1.8	0.022	—	—	—	—	—	—
215	—	—	—	—	—	—	10.6	—	—
230	38.6	0	—	—	—	—	—	—	—
Mean k (15–170 min.) = 0.0235				Mean k = 0.0555			Mean k (5–65 min.) = 0.051		
Mean k /c.c. methyl alcohol = 0.0117				Mean k /c.c. methyl alcohol = 0.0139			Mean k /c.c. methyl alcohol = 0.0127		

From this table it can be readily seen that the resin of *D. malaccensis* reacts to caustic potash and methyl alcohol in a very similar way to the "Sumatra-type" resin. There is the same reduction in the rotation on adding methyl alcohol and the same conversion from laevo- to dextro-rotation on the addition of caustic potash in methyl alcohol to its benzene solution, and a subsequent decline in dextro-rotatory power with time. It would appear too as if increasing the methyl alcohol accelerated the

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decline. The magnitude of the initial change-over from laevo- to dextro-rotation is proportionately less than in the "Sumatra-type".

The results of adding caustic potash in methyl alcohol to the resin of *D. elliptica* and to rotenone in solution in benzene are given in Table IV. It will be observed here that although, as would be expected, the addition of methyl alcohol reduces the specific rotation, the addition of caustic potash in methyl alcohol does not produce the conversion to dextro-rotation, although it causes an immediate drop in the laevo-rotatory power, any subsequent change being probably not outside experimental error.

Table IV

The effect of adding caustic potash in methyl alcohol to benzene solutions of the resin of D. elliptica, and of rotenone

Description	Resin from <i>D. elliptica</i>			Rotenone	
	Dilution	E.D.	E.C.	*	†
Dilution in benzene g./100 c.c.		2.080	2.089	0.900	0.432
=g. root/100 c.c.		12	24	—	—
α_D^{20}		-2.05°	-2.05°	-2.19°	-1.01°
$[\alpha]_D^{20}$		-98.4°	-98.4°	-228°	-234°
Methyl alcohol added to 5 c.c. of above		2 c.c.	4 c.c.	4 c.c.	4 c.c.
Conc. after addition of methyl alcohol g./100 c.c.		1.492	1.161	0.533	0.240
$[\alpha]_D^{20}$ after adding methyl alcohol		-85.1°	-80.1°	-214°	-215°
$[\alpha]_D^{20}$ for equivalent conc. in benzene from graph		-99°	-99.5°	-232°	-233°
G.-equivalents of caustic potash added		1	2	2.1	5.3
$[\alpha]_D^{20}$ 5 min. after mixing		-9.38°	-0.86°	-79°	-79°
$[\alpha]_D^{20}$ after time in brackets		-0.67°	-2.58°	-68°	-71°
		(105 min.)	(100 min.)	(2731 min.)	(1112 min.)

* Rotenone of equivalent strength to that present in *D. elliptica* resin E.C. Methyl alcohol and caustic potash as in E.C.

† Rotenone of equivalent strength to that present in *D. malaccensis* resin M.C. Methyl alcohol and caustic potash as in M.C.

Many other concentrations of rotenone in benzene ranging from 5 g. to 1 g. per 100 c.c. were tested with various amounts of methyl alcohol and caustic potash. The addition of methyl alcohol reduced the rotation slightly and caustic potash very materially and there was in the latter case a slight further loss with time, but in none did the rotation become positive. There is a tendency on the addition of caustic potash for crystalline matter to be precipitated if methyl alcohol is not present in considerable amount.

It is clear from these figures that the "Sumatra-type" and *D. malaccensis* resins contain ingredients that are not present to any extent in the sample of *D. elliptica* used. Rotenone, from the data given in Table IV, obviously plays no part in this characteristic switch-over in the rotation, and it is highly probable, from the results obtained with *D. elliptica*, that deguelin has no part in it either. Since the resins of the "Sumatra-type" and of *D. malaccensis* are distinguished from that of the *D. elliptica* by the large proportion of material extracted from

their ether extracts by caustic potash, the fractions obtained in this way were tested. They were dissolved in benzene and caustic potash in methyl alcohol was added, the amount of caustic potash added being based upon an equivalence to toxicarol. The results are given in Table V.

Table V

Effect of addition of caustic potash in methyl alcohol upon benzene solutions of fractions from "Sumatra-type" resin

Material extracted and precipitated by caustic potash. Conc. of solution = 3.234 g./100 c.c. $\alpha_D^{20} = -1.63^\circ$. $[\alpha]_D^{20} = -50.4^\circ$.				Material soluble in ether after extraction by potash. Conc. of solution = 3.018 g./100 c.c. $\alpha_D^{20} = -2.79^\circ$. $[\alpha]_D^{20} = -92.4^\circ$.				
2 c.c. methyl alcohol added to 5 c.c.			4 c.c. methyl alcohol added to 5 c.c.			4 c.c. methyl alcohol added to 5 c.c.		
Conc. after addition of methyl alcohol = 2.374 g./100 c.c.			Conc. after addition of methyl alcohol = 1.797 g./100 c.c.			Conc. after addition of methyl alcohol = 1.677 g./100 c.c.		
$[\alpha]_D^{20}$ after addition of methyl alcohol = -22.8° .			$[\alpha]_D^{20}$ after addition of methyl alcohol = -6.1° .			$[\alpha]_D^{20}$ after addition of methyl alcohol = -75° .		
Equivalents of potash added. Resin calculated as having mol. wt. = 410				Equivalent of potash added assuming mol. wt. = 394				
1				1				
Solution becomes very dark after addition of potash.								
$[\alpha]_D^{20}$ = approximately -12° .								
Little or no change in readings after 30 min.								
Time min.	$[\alpha]_D^{20}$	$[\alpha]_{k_1-210}$	k	$[\alpha]_D^{20}$	$[\alpha]_{k_1-226}$	k		
1	+206	206	—	+255	195	—		
2	251	191	0.075	240	180	0.080		
5	237	177	0.038	212	152	0.062		
10	213	157	0.030	178	118	0.056		
15	196	136	0.030	153	93	0.053		
20	181	121	0.028	131	71	0.053		
25	169	109	0.0265	115	55	0.053		
30	157	97	0.026	104	44	0.051		
35	145	85	0.026	92	32	0.053		
40	137	77	0.025	86	26	0.052		
45	128	68	0.025	80	20	0.052		
50	120	60	0.025	—	—	—		
55	115	55	0.024	—	—	—		
60	111	51	0.024	70	10	0.050		
75	96	36	0.024	65	5	0.050		
90	85	25	0.024	—	—	—		
105	78	18	0.023	62	2	0.044		
120	72	12	0.024	—	—	—		
150	65	5	0.025	—	—	—		
165	—	—	—	61	1	0.032		
180	62	2	0.026	—	—	—		
210	60	0	—	—	—	—		
225	—	—	—	60	0	—		
Mean k ($t = 10-90$ min.) = 0.026				Mean k ($t = 10-75$ min.) = 0.053				
Mean k /c.c. methyl alcohol = 0.013				Mean k /c.c. methyl alcohol = 0.013				

Solution becomes very dark after addition of potash.
 $[\alpha]_D^{20}$ = approximately -12° .
Little or no change in readings after 30 min.

The resin recovered from the material extracted and precipitated by caustic potash has been shown in Part II(5) of this series (p. 894) to be laevo-rotatory when dissolved in benzene. Its benzene solution reacts to the addition of caustic potash in methyl alcohol in a similar way to

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the resin of the "Sumatra-type" and of *D. malaccensis*. An instantaneous change-over to dextro-rotation takes place followed by an approximately unimolecular decline of the specific rotation with time. The magnitude of the initial change is, however, greater than it is with the crude resins.

The material left in the ether layer after extraction with caustic potash reacts more like *D. elliptica* and rotenone, in that there is a reduction in the laevo-rotatory power but no change of sign.

The separation of the fraction extracted and precipitated by caustic potash enabled us to test further the effect of methyl alcohol in accelerating the decline in the rotation after the addition of caustic potash. It will be seen from Table V and from Fig. 3, section II, that when the proportion of alkali to this resin is kept constant but the amount of methyl alcohol added to its solution increased twofold the rate of decline is practically doubled.

A repetition of the experiment with the potash extracted compound confirmed our view that the accelerating effect of methyl alcohol depended more on the proportion of the two solvents than on that of methyl alcohol to the resin. Three quantities of the "toxicarol" resin were weighed out and dissolved in benzene. The concentrations in the mixtures used are set out together with the mean velocity coefficients in Table VI.

Table VI

	Conc. in meth. alcohol g./100 c.c.	Benzene c.c.	Methyl alcohol c.c.	KOH g. equiv.	Mean <i>k</i> (10-60 min.)	Mean <i>k</i> (whole of reaction)
(1)	2.286	5	2	1	0.022	0.020
(2)	2.286	5	4	1	0.048	0.047
(3)	1.143	5	2	1	0.025*	0.026

* This value becomes 0.022 if the final rotation at which the action comes to a standstill is taken as equal to that for Nos. 1 and 2.

If the effect depended on proportionality of methyl alcohol to resin we should expect the mean velocity coefficient of No. 3 to be on the higher level of No. 2, but if on that of the two solvents on the lower one of No. 1. The mean value for No. 3 is on the lower level.

It should, however, be pointed out that during the first 5 min. the reaction in the case of No. 3 was more rapid than in No. 1 and approximated to that of experiment No. 2, but its rate rapidly fell and for the next 50 min. was nearly the same as No. 1. The further decline in the rate of reaction was steady, and finally the velocity coefficient became less than 0.02.

In order to make it easier to compare the results tabulated in

Tables II-V, we have graphed the data in the sections of the Figs. 2, 3 and 4. In Figs. 2 and 3 the specific rotations, and in Fig. 4 the logarithms of the specific rotations minus the specific rotation at which the reaction approaches a standstill, are plotted against time in minutes.

Fig. 2, section I, illustrates the effect of increasing the amounts of caustic potash upon the induced dextro-rotation of the "Sumatra-type" resin. There is little or no alteration in the rate of decline whether 1, 2

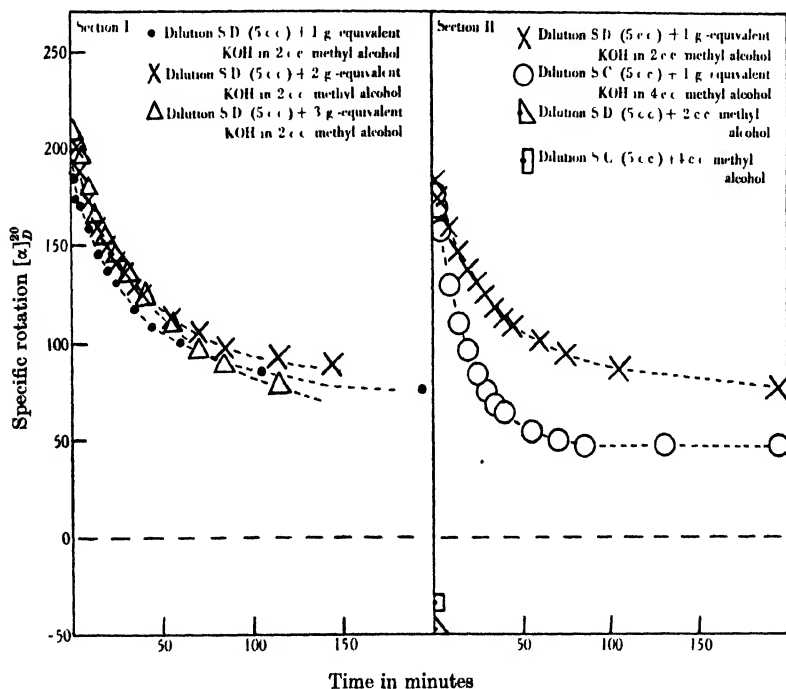


Fig. 2. Decline in the alkali-induced dextro-rotation of benzene solutions of the "Sumatra-type" resin. Section I, effect of concentration of potash. Section II, different concentrations of resin and methyl alcohol.

or 3 g.-equivalents of potash are used. Fig. 2, section II, illustrates the difference in the rate of the decline of the induced specific rotation of different initial concentrations of the "Sumatra-type" resin in benzene. This change in rate is due, however, to the different amounts of methyl alcohol added with the caustic potash in the two cases. Fig. 3, section I, gives curves illustrating the decline in the induced dextro-rotation of the resin of the "Sumatra-type" and *D. malaccensis* as contrasted with that of *D. elliptica*, which remains laevo-rotatory, after treatment with equivalent amounts of potash in the same quantities of methyl alcohol.

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Fig. 3, section II, shows the effect of increasing the methyl alcohol upon the rate of the decline of the induced dextro-rotation of the benzene solution of the "toxicarol" resin. The velocity coefficient of the reaction is practically doubled by doubling the proportion of methyl alcohol to benzene solution of resin.

The semi-logarithmic graphs in the sections of Fig. 4 are intended to give a comparative conspectus of the relative rates of decline of the induced dextro-rotations on the addition of alkali in methyl alcohol to the benzene solutions of the resins of the "Sumatra-type" root, *D. malac-*

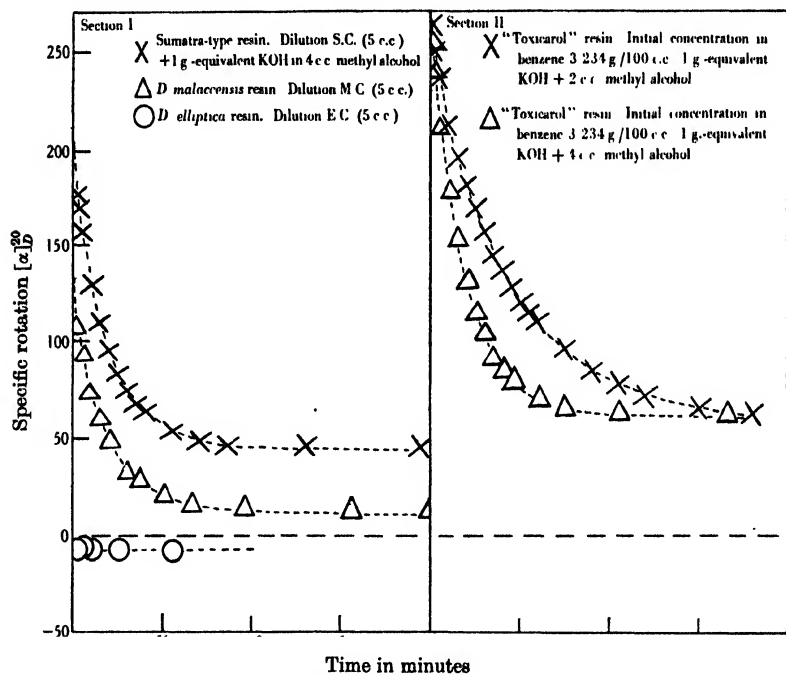


Fig. 3. Effect of adding caustic potash in methyl-alcohol upon the rotations of three types of derris resin and upon "toxicarol" resin (in solution in benzene).

censis, and the substance extracted and precipitated from their ether solutions by caustic potash ("toxicarol" resin). The effect of doubling the volume of methyl alcohol added with the caustic alkali is rendered evident by the steeper slope of the lines. That side reactions are taking place is evidenced by the fact that in several cases the points are only fitted by a straight line over a limited period. It is indeed rather surprising that the semi-logarithmic graphs approximate so closely to straight lines in so many instances, when the readiness with which some of the compounds, present in derris root, undergo chemical change in

the presence of alkali is considered. Indeed, most of the resins gradually darkened in colour after the addition of alkali until finally the benzene-

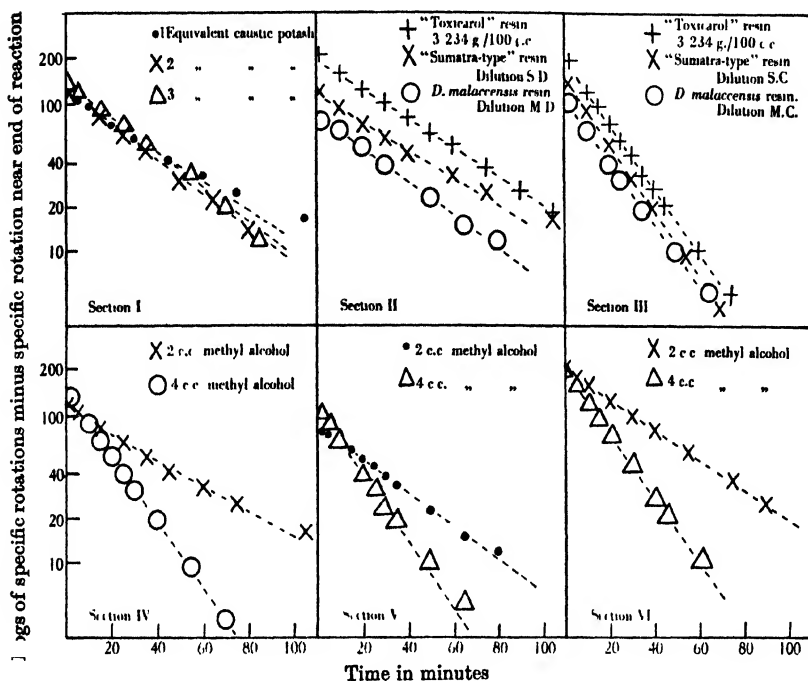


Fig. 4. Decline of induced dextro-rotation with time (semi-logarithmic scale).

Section I. "Sumatra-type" resin dilution S.D. in benzene with different amounts of caustic potash added in same quantity of methyl alcohol.

Section II. 5 c.c. benzene solutions of three resins + 1 g. equiv. caustic potash + 2 c.c. methyl alcohol.

Section III. 5 c.c. benzene solutions of three resins + 1 g. equiv. caustic potash + 4 c.c. methyl alcohol.

Section IV. 5 c.c. benzene solutions of "Sumatra-type" resin with 1 g. equiv. caustic potash and different amounts of methyl alcohol × dilution S.D. O dilution S.C. (p. 905).

Section V. 5 c.c. benzene solutions of *D. malaccensis* resin with 1 g. equiv. of caustic potash and different amounts of methyl alcohol • dilution M.D. Δ dilution M.C. (p. 907)

Section VI. 5 c.c. benzene solutions of toxicarol resin with 1 g. equiv. of caustic potash and different amounts of methyl alcohol. Initial concentrations in benzene were the same (3.234 g./100 c.c.). (p. 909).

methyl alcohol solutions were of such a deep red shade that accurate polarimeter readings were impossible. In addition, it is known that the specific rotations of the resins and of rotenone increase linearly with decline in concentration (Fig. 1); it is possible that a similar effect may

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characterize the dextro-rotatory compound, and thus the rotations observed during the reaction may not have exactly the same equivalence as a measure of the concentrations of the reacting substance.

CONCLUSIONS

Owing to the large variations in type characterizing derris root, as many chemical and insecticidal data as possible are required about its different varieties. It seems to us that only in this way can the existing confusion be cleared away, or accurate information be made available as to the effect of genetical, soil, or climatic factors upon the quality of these plants. It is possible that in the course of time only a few varieties or types will be produced under cultivation and thus the problem of the assessment of their value be simplified; but at present there is little assurance that consignments as they come on to the market can be put into any one category as far as the chemical characteristics, botanical make-up and insecticidal potency are concerned. The evaluation by chemical means is one of great difficulty and is rendered more so by this contingency. The fractionation therefore of the resins of derris and cubé and the determination of the chemical characteristics and insecticidal potency of the fractions appears to be a matter of some urgency if the methods of assessment are to be improved.

In the foregoing section a study has been made of the optical activities of the benzene solutions of resins and their fractions derived from three samples of derris root containing approximately the same amounts of ether extract. They were *D. elliptica*, *D. malaccensis*, and the "Sumatra-type" of Cahn & Boam. They were all laevo-rotatory, the specific rotations being in descending order of magnitude *D. elliptica*, *D. malaccensis*, the "Sumatra-type". The rotations did not give a quantitative measure of the relative toxicities of the roots to *Aphis rumicis*, nor was it possible to obtain a quantitative assessment of the relative toxicities by an examination of the rotations of their fractions.

The addition of methyl alcohol to solutions of rotenone and of the resins reduced their specific rotations. A preliminary attempt to fractionate the resin of the "Sumatra-type" by means of methyl alcohol gave a fraction which was dextro-rotatory in solution in methyl alcohol but laevo-rotatory in benzene. The resins of the "Sumatra-type" and of *D. malaccensis* when dissolved in benzene and treated with a standard solution of caustic potash underwent a change of sign in their optical rotation. There followed a decline in the rotation on standing, the time reaction being approximately unimolecular in type although side reac-

tions apparently accompanied it. The speed of the decline was only slightly affected by the amount of caustic potash added or by the amount of resin present, provided the amount of methyl alcohol added to the same volume of solution was the same. An increase in the amount of methyl alcohol added with the caustic potash accelerated the decline in activity. In our experiments the rotations never reached zero after the initial change of sign. Rotenone and the resin of *D. elliptica* tested did not undergo a change of sign under these conditions, but the normal laevo-rotation was reduced instantaneously and then either slowly declined or remained approximately constant. The "Sumatra-type" root was therefore much more like *D. malaccensis* than *D. elliptica*.

When the ether solutions of the "Sumatra" or *D. malaccensis* resins were treated with caustic potash, a portion was extracted and precipitated by the alkali and a portion remained in solution in the ether (see Part II of this series, p. 887)(5). The yellow precipitate, on acidification and extraction, gave a resinous product¹ which was laevo-rotatory in benzene solution but underwent the instantaneous change of sign when caustic potash was added in methyl alcohol. The rotation decreased on standing and the velocity of this reaction was also accelerated by an increase in the amount of methyl alcohol added with the caustic potash. The fraction soluble in ether behaved like the resin extracted from *D. elliptica*, showing no change of sign of rotation on the addition of caustic potash in methyl alcohol to a benzene solution. It appears probable that the material extracted from ether solutions of the "Sumatra-type" or *D. malaccensis* contains the precursor of toxicarol in high concentration.

Further work on these lines is required in order to ascertain the validity of the reaction as a diagnostic sign and to discover whether rotation methods would be of use in ascertaining the amount of "toxicarol" present in a sample of roots and if its presence is determined by genetical, climatic or cultural conditions.

SUMMARY

A study has been made of the rotations of the resins from three types of derris root, and of a fraction rich in toxicarol separated from two of them. No strictly quantitative relationship between their rotations and their toxicities to *Aphis rumicis* has been found. The addition of caustic

¹ Since this paper was sent to press a crystalline derivative, showing the characteristic change of sign in rotation on the addition of caustic potash in methyl alcohol to its benzene solution, has been isolated from this resin.

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potash in methyl alcohol to the benzene solutions of the resins induces a characteristic change from laevo- to dextro-rotation in the samples rich in toxicarol. The induced dextro-rotation then declines in value with time. This effect is shown by the "toxicarol" resin. The rate of the decline is accelerated by increasing the amount of methyl alcohol.

We are greatly indebted to the Copper Technical Bureau for gifts of the "Sumatra-type" root and of resins derived from this strain.

We wish to express our thanks to Mr C. Read for help during the course of this work.

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MODERN DEVELOPMENTS IN RESEARCH ON INSECTICIDES. PART I. GENERAL SURVEY*

By F. TATTERSFIELD

The modern era of insecticide research began in 1912-16 when Shafer (Michigan Agric. Exp. Sta. Tech. Bull., 1912, No. 11; 1916, No. 21) published his papers on "How insecticides kill." To-day, it is true, one would find that some of his conclusions needed correction, but they have stimulated and encouraged enquiry into the more fundamental aspects of insect toxicology, and they undoubtedly rank as classics. Important papers were published between 1910 and 1915 by Vermorel and Dantony (Compt. rend., 1910, 151, 1144; 1911, 152, 972; 1912, 154, 1300), by Lefroy (Ann. Appl. Biol., 1914, 1, 280), and by Cooper and Nuttall (J. Agric. Sci., 1915, 7, 219) in which the importance of the physical characteristics of spray fluids, particularly surface tension, as affecting wetting and spreading were analysed and one may say that the attempt to bring greater precision into insecticide and fungicide investigations had begun. At that time, some of the more important insecticides and fungicides, the arsenicals, nicotine, derris, pyrethrum, lime sulphur, Bordeaux and Burgundy mixtures, were known and some of them in use. Much of the work since then has been devoted to the study of these and allied products in order to discover their range of usefulness, to increase their efficiency, and to find more powerful substitutes, safer to man, domestic animals, and the plant.

Laboratory studies

In the control of insect pests performance in the field is the final court of appeal, but to extend the range of research, laboratory methods of assessing toxicity and the factors contributing to it were necessary. Insecticides are divided usually into two main classes: (a) contact poisons with which are frequently ranked fumigants, sometimes termed respiratory poisons; (b) stomach or internal poisons. Class (a) includes those poisons which, having been brought into external contact with the insect, penetrate its integument and kill by reaching some vital part. Class (b) act by being ingested with the food.

The forms of laboratory apparatus used for testing insecticides are associated with the names of Tattersfield and Morris, O'Kane and his co-workers, F. L. Campbell, H. H. Richardson, Peet and Grady. The methods of these workers aim at atomising known

amounts of spray as evenly as possible over insects on a constant plane area or flying in a constant volume. It is assumed that the insects will receive an amount of spray which is correlated with their size. The tests range over a number of concentrations. After spraying, the insects are set aside, observed over a period of some days, and graded for the effect produced after a definite period of time. The effects may be plotted to give so-called dosage-mortality curves.

Other methods have been explored. O'Kane and his co-workers (New Hamps. Tech. Bull. No. 54, 1933) applied minute droplets to different areas of the common meal-worm and found that the sensitivity of reaction varied with the position of the point of application. With the knowledge so gained, they elaborated a new technique for the initial appraisal of insecticides, in which minute droplets of constant size were applied to three well-defined points, and the potency of the compounds was judged by the time taken to produce a convulsive effect. The results were compared with those obtained by spraying. Nelson and his co-workers (Soap, 1934, 10, No. 10, 85) have elaborated a similar method in which they pipette known minute amounts of the insecticide, dissolved in absolute ethyl alcohol, on the centre of the ventral surface of the thorax of insects which have been reduced to quiescence by chilling; house flies reared under carefully controlled conditions are mainly used. After treatment, the flies are set aside with food and water in screened dishes and the effects judged after a twenty-four hour period.

Methods depending on dipping insects, usually enclosed in cages, into different concentrations of insecticide have also been used. The conditions have to be carefully controlled and there is always a risk of stomach poison effects invalidating the interpretation of the results.

Injection methods have also been explored (J. Gen. Physiol., 1926, 9, 433). In this way F. L. Campbell (J. Econ. Entom., 1932, 25, 905) found that certain of the basic dyes (malachite-green in particular) were toxic to silk worms. Hockenyos and Lilly (*ibid.*, 253) also used an injection method and were unable to find that there was a direct proportionality between susceptibility and size. Injection methods present considerable possibilities in exploring the fundamental reactions of insecticides but they are too remote from procedure which can be adapted in practical work to make the results easy of a utility interpretation.

* Read before a Joint Meeting of the London Section and the Food Group on December 7, 1936.

Stomach poisons

Means of assessing toxicity

The critical study of stomach poisons dates back a number of years but the mode of assessing results has been variable. They can be summarised thus: Holloway (J. Econ. Entom., 1912, 5, 452), poison exponent, time to kill by Paris green ÷ time to kill by new poison. Scott and Siegler (U.S. Dept. Agric. Bull. 278, 1915), area of poisoned leaf consumed and time taken to kill. Tartar and Wilson (J. Econ. Entom., 1915, 8, 481), determination of arsenic in dead insects. Lovett and Robinson (J. Agric. Res., 1917, 10, 199), arsenic acid in body ÷ arsenic acid in excreta, together with time taken to kill.

W. Moore (Univ. Minn. Tech. Bull. No. 2, 1921) working with the arsenicals compared several of the methods. Thus he determined: (1) weight of poisoned bait consumed before death, (2) average number of hours necessary to produce death, (3) ratio of chemicals in tissues to that in excreta (lead arsenate = 1). Moore selected the last method as being most suitable for evaluating the toxicity of the arsenicals and it unquestionably does enable one to ascertain important facts such as the more rapid absorption of acid lead arsenate than of basic lead arsenate (Tartar and Wilson; Lovett and Robinson), but as a means of determining relative toxicity or of comparing a miscellaneous list of chemical compounds, it would not only prove excessively laborious but unless coupled with information of the amounts of poison administered might lead to no information of economic importance.

F. L. Campbell (J. Econ. Entom., 1930, 23, 357) considered several methods of evaluating stomach poisons and concludes that no method measures at all accurately the relative toxicities of compounds unless the dose administered is determined, and he makes the pertinent comment that "the speed of toxic action of a stomach poison is determined by a whole chain of physical and chemical events occurring from the time the poison enters the stomach until the insect dies and the rate of the chain of events is dependent on the size of the dose and not on some fraction of the dose responsible for the death of the insect." As a result of this work he finally decided that for dusts the most practical method was to deposit in a specially devised apparatus an ascertainable and evenly distributed weight of poison on discs of leaf of constant size. Sandwiches were prepared by superimposing a second circle of leaf. The amount taken by the insect could be ascertained by measuring the unconsumed leaf area by a planimeter or, as Bulger has suggested, by photoelectric methods. The time to paralyse and to kill and its inverse the speed of toxic action as well as the dosage are determined. The difficulty with this method unquestionably is that for insecticides mixed with some diluent there is a danger that the deposit on the leaf may not be of the same composition as the original dust. This necessitates separate operations with the poison and the diluent. The method has been slightly modified by Stellwaag and by Bulger and it has proved a powerful instrument of research.

Other methods depending on the consumption of poison baits of various kinds and, in the case of codling moth larvæ, of plugs of apple have also been used.

There are two ways of expressing the toxicity results obtained: (a) dosage may be compared with per cent. mortality or (b) dosage with the time taken for a lethal effect to take place or with its reciprocal, "the speed of toxic action."

Some of the more simple of the statistical transformations have been of value in enabling the comparative toxicities of various compounds to insects to be assessed. It is a matter of some interest that in both drug and insecticide work similar methods were independently devised for simplifying the quantitative expression of the results. O'Kane and his co-workers and C. I. Bliss were almost contemporaneous with Hemmingens and Gaddum.

When the concentration of poison is plotted against the percentage kill an asymmetrical or skew S-shaped curve usually results. It is fairly obvious that different portions of such curves vary in validity for estimating toxicity. Thus the 100% mortality is approached asymptotically and cannot be accurately defined. R. A. Fisher in a note appended to an early paper of Tattersfield and Morris (Bull. Entom. Res., 1924, 14, 223) pointed out that comparisons were statistically sounder when made at concentrations giving a mortality of 50%, the so-called median lethal dose. Although McCallan and Wilcoxon (Contr. Boyce Thompson Inst., 1933, 5, 173) have expressed doubt as to the complete validity of this generalisation, it has been very generally used. It is obviously not satisfactory to be bound down to so narrow a limit, not always easy to determine, and, moreover, not meeting the needs of the practical man, who wishes to know concentrations capable of killing a much higher figure and if possible 100% of his pests. These sigmoid curves which owe their shape, mainly, to variations in the susceptibility of individual insects to the action of the poison, can be converted into straight lines by certain statistical devices. The methods elaborated by O'Kane and his co-workers (New Hamps. Agric. Tech. Bull. Nos. 39 and 58) eliminate the variations in susceptibility, by plotting dosage-mortality results on probability paper and projecting the data on an arbitrary arithmetic scale, the logarithms of the values so obtained when plotted against the logarithms of the concentrations giving straight lines. C. I. Bliss (Ann. Appl. Biol., 1935, 22, 134, 307) plots the logarithms of the actual dosages against dosages inferred from the integrated normal frequency distribution. These inferred dosages expressed in terms of the standard deviations he has entitled "probits." By Bliss' method the converted dosage-mortality curves often show a break in the straight line for concentrations of insecticide killing only a small proportion of the insects. O'Kane has pointed out that such a break would not occur were the logarithms of probits taken.* Bliss (J. Econ. Entom., 1935, 28, 646), however, maintains that the logarithm of a probit is not a natural function and that the break probably indicates that low concen-

* The relation between the method of Bliss and O'Kane is that Bliss plots the probits "p" against log concentrations, whereas O'Kane plots log (18.44p-17.22) against log concentration. For a full appreciation of these methods the original papers should be consulted.

trations of insecticides act in a different way from high concentrations, also that the change of slope may have a biological reality. The main features about these mathematical conceptions are that they simplify the comparison of insecticidal values, enable a larger number of data to be taken into account, and give a means of arriving at the approximate concentration of an insecticide giving a 100% mortality.

The estimation of the time of survival and its reciprocal (the speed of toxic action) has been extensively used in research. Reference should be made to Clarke's monograph "The Mode of Action of Drugs on Cells" (Arnold) for an analysis of this relationship. F. L. Campbell (J. Gen. Physiol., 1926, 9, 727; J. Agric. Res., 1926, 32, 356) has illustrated its use for the evaluation of stomach poisons by plotting the speed of toxic action against the dosage and by determining the areas enclosed beneath the curves and taking their ratios. This is equivalent to taking the ratios of the sums of the respective speeds of toxic action over a whole range of dosages. The method, however, may possibly lead to some misinterpretation if the comparison is made between compounds of very different physiological action, as some insecticides are slow but cumulative and others rapid but evanescent in their effects. In evaluating stomach poisons it is customary in critical studies to state the dosage per body weight but, although in general small larvae are less resistant than large larvae, there is some evidence to show that the toxic effect is not strictly proportional to the size of the insect. In the case of contact insecticides it is assumed that the amount of an insecticide at constant concentration sprayed on an insect is proportional to its size. Sufficient data have not yet been accumulated to give anything like a final answer as to what power of the body weight should be taken. Bliss (J. Exp. Biol., 1936, 13, 95), who has analysed some of Campbell's data for sodium arsenate, considers the logarithm of the speed of toxic action to be preferable to the rate of toxic action itself for assessing toxicity and found the data for all the instars of the insect used (silkworm) to fall on one straight line when log rate was plotted against the log of the dose adjusted by the size factor $W^{1.6}$.

Field trials

The quantitative assessment of results in the field has now largely taken the place of the general impression gained on inspection. It is, however, almost impossible here to deal in even the slightest detail with the methods used. Illustrations of these are given by A. M. Massee (Ann. Appl. Biol., 1926, 13, 452) and by Bartlett (Supplmt. J. Roy. Statist. Soc., 1936, 3, 189). Fisher's statistical methods (particularly the analysis of variance), for which comparatively small samples may be used, have enabled workers to estimate the significance of their results without being involved in almost insuperable labours.

Chemical developments

In the whole of this work there has been a fairly close collaboration between chemist, entomologist, physi-

cist, and statistician. One should not, however, omit from this list those who are actively engaged in industrial chemistry who have presented a large array of products to be tested and who have been able to draw on experience from other fields of work.

Tar distillates.—The introduction from Holland of the tar-distillate washes for spraying dormant fruit trees a few years after the war brought about a revolution in spraying practice and much discussion raged around them for some years. Tutin (Long Ashton Repts., 1927, 81) showed the great insecticidal importance of the high-boiling (280–360°) neutral compounds of vertical-retort tar and industrial chemists provided new means of emulsification which met the difficulties found in using the early products with hard water. It was soon noted that an increase in the incidence of red spider resulted from the use of these washes, and to meet this and to extend their range of usefulness heavy petroleum oils were added. It is a matter of considerable interest that the tar products should be lethal to aphid eggs and petroleum, although said to be somewhat uncertain to those of the red spider. The time of application of these products is of course a matter of importance as a certain amount of the bud damage may result from spraying too late in the season. Tar distillates are therefore used as winter washes on dormant trees. Petroleum washes, however, are used for both winter and summer spraying but for the latter purpose they must contain a comparatively low proportion of unsaturated hydrocarbons.

Petroleum oils.—The use of petroleum as an insecticide dates back a considerable time. Kerosene and a heavier fraction "solar distillate" were applied to trees by Pickering but the earlier petroleum derivatives while proving insecticidal frequently led to serious phytocidal effects. The so-called heavy oils when first tested had a slower but none the less pronounced injurious action. This acute toxicity was traced down to the sulphonatable fractions and highly refined oils were produced for spray purposes. These highly refined heavy petroleum oils came into such extensive use that Woodworth (J. Econ. Entom., 1930, 23, 848) thought that the prospects were that they would become the chief insecticides. Although that promise is not quite so certain to-day, they undoubtedly occupy one of the foremost places. Petroleum oils of various types, particularly as dormant sprays, are used extensively in the United States for the control of scale insects. In the citrus-growing areas of Florida and California very large amounts of oil sprays have been used annually, two or three applications being made each year. In America and in England highly refined oils have been shown to be very useful as acaricides, but the application in America of highly refined oils in combination with or following lead arsenate for the control of codling moth has not been generally adopted, except in regions where great difficulty in its control has been met with. Their employment increases the difficulty of the subsequent washing of the fruit.

The excessive use of even highly refined saturated oils appears to be causing some uneasiness. Knight, Chamberlin, and Samuels (Plant Physiol., 1929, 4, 299) and Rohrbach (*ibid.*, 1934, 9, 699) mention a number of manifestations of chronic injury that have been observed after their application. There appears too to be some

of cumulative damage which may, however, be unfounded. The metabolic disturbances observed in the tree are apparently due to physical rather than chemical causes and result from the intrusion of the oil, particularly heavy oil, into plant tissues. Kelley (Illinois Agric. Bull., 1930, No. 348) considered the injury to be accentuated by high humidity and that highly refined oils are not a guarantee against it, but drought conditions may play an important part. Further research may overcome these difficulties and it is generally recognised that highly refined petroleum oil emulsions are amongst the most powerful controlling agents of insect pests, particularly those of citrus trees. Their importance for use in this connexion has been accentuated by the appearance of scale apparently more resistant than usual to hydrocyanic acid, and as the damage is probably partly due to overspraying it would appear that the incorporation of highly toxic chemicals with the petroleum may well lead to a solution of the difficulty.

The acute phytocidal effect, in contrast with the chronic effect mentioned above, of crude petroleum oils is largely due to the presence of unsaturated hydrocarbons and compounds extracted on sulphonation. In a recent paper by Tucker (Ind. Eng. Chem., 1936, 28, 458) it is claimed that the toxicity of these compounds is largely due to the formation by oxidation of asphaltogenic acids and that the unsaturated compounds are not themselves toxic in a chemical sense. Young (J. Agric. Res., 1934, 49, 559) considers that oils sulphonated by sulphur dioxide are more dangerous than those sulphonated by sulphuric acid; thus the chemical nature of these residues is an important consideration.

It is thus obvious that compounds like the tar distillates and petroleum spray oil derivatives, obtained as they are from diverse raw materials emulsified in various ways, are not easy to standardise. One of the most important papers we have on this subject we owe to Hubert Martin (Ann. App. Biol., 1935, 22, 334). It is much too detailed to summarise here, but he divides these oils and their preparations into various grades according to the uses for which they are intended and gives a variety of chemical and physical criteria with which each grade should comply, in the hope that they will be tested out in practice and meet with the consideration of those who have an interest in them.

Nicotine and related products.—When it is borne in mind that this alkaloid is toxic both as a contact and as a stomach poison and kills in the vapour phase, and that there is little immunity to its effect on egg, larvæ, or adult stages of insects, it is not surprising that it still holds an important place to-day as an insecticide. Some rather disagreeable properties and its high cost react against its use and substitutes have been actively sought. It has come recently again into great prominence in America, where an almost fervid campaign for substitutes for the arsenicals is being pursued, particularly for the control of codling moth on apples. The volatility of nicotine, the fact that it is not permanent enough after spraying, and its cost have acted as limiting factors to its use. Loss of activity after spraying seems likely to be overcome, and products like nicotine oleate and humate as well as nicotised peat have been prepared. The most interesting of these products is nicotine-

bentonite, which seems already to have had some success in controlling codling moth. Although in field trials it may not as yet have come up to lead arsenate in performance (J. Econ. Entom., 1936, 29, 590), it shows considerable promise (*ibid.*, 369).

The high cost of nicotine has led to the search for substitutes, and research workers turned to compounds of similar structure and this finally led to the synthesis of dipyrilidyl, pyridylpiperidines, and dipiperidines. It was found (C. R. Smith, C. H. Richardson, and H. H. Shepard (*ibid.*, 1930, 23, 863, and other references) that 3-pyridyl-2'-piperidine (neonicotine) was of the same order of toxicity as nicotine. There are important relationships between chemical constitution and insecticidal action among this group of compounds, but it is interesting to note that amongst the pyridylpiperidines the most toxic is of a similar structure to nicotine, 2-pyridyl-3-piperidine being less toxic. Shortly afterwards the alkaloid anabasine, isolated from *Anabasis aphylla*, was found by Russian workers to have a powerful contact action and the work of Orekhov and Menschikov (Ber., 1931, 64, 266) and of C. R. Smith (J. Amer. Chem. Soc., 1932, 54, 397) showed it to be identical in structure with neonicotine, but optically active. Lævorotatory anabasine has been found to have, if anything, a higher order of toxicity by contact to aphides than nicotine but it is a less powerful stomach poison. The spatial arrangement of these compounds seems to play an important part in toxicity and it is of considerable interest that Craig and Richardson (Iowa State Coll. J. Sci., 1933, 7, 477) have found racemic nicotine to be much less toxic than the natural lævo-alkaloid, and Starr and Richardson (J. Econ. Entom., 1936, 29, 214) state that the toxic action of the lævo-form of 2-p-tolylpyrrolidine tartate differs in type from that of the dextro-form. Recently too, Richardson, Craig, and Hansberry (J. Econ. Entom., 1936, 29, 850) have demonstrated interesting contrasts between the toxicities of the α - and the β -nicotines and normicotines, the β -compounds being in every case much more toxic than the α -compounds.

Organic thiocyanates.—One of the most interesting of recent discoveries in this field is the high toxicity of the organic esters of thiocyanic acid, products of industrial research. It has long been known that allylthiocarbimide was an extremely potent insecticide. The first announcement of these products as far as can be traced came in 1932, when Murphy and Peet (J. Econ. Entom., 1932, 25, 123) showed that a certain aliphatic thiocyanate had a high toxicity to aphides. The compound reported as β -butoxy- β' -thiocyanodiethyl ether was shown to be highly toxic to a variety of insect pests. Later, lauryl thiocyanate was shown also to have a high toxicity and to have a large range of applicability for pest control. Wilcoxon and Hartzell (Contr. Boyce Thompson Inst., 1934, 6, 269; 1935, 7, 29) have made a careful study of this group of compounds and found that trimethylene dithiocyanate, $\text{CNS-CH}_2\text{CH}_2\text{CH}_2\text{-CNS}$, and phenyl γ -thiocyanopropyl ether, $\text{C}_6\text{H}_5\text{-O-CH}_2\text{CH}_2\text{CH}_2\text{-CNS}$, are highly insecticidal but also have some phytocidal effect, but this varied with the plant, many plants showing no injury. They were ineffective as stomach poisons. Wilcoxon and Hartzell report that on a basis of SON content the

methylene derivative was less toxic than lauryl thiocyanate but that the latter was not so toxic as rotenone. Bousquet, Salsburg, and Dietz (Ind. Eng. Chem., 1935, 27, 1342) show an interesting relationship between the straight-chain thiocyanates and toxicity, a rise in toxic value being observed as the number of carbon atoms in the homologues increase up to 12, which was followed by a decline. This result is of the same type as that obtained at Rothamsted for the fatty acids, except that the decline came after the 11-carbon atom acid in that case. Recent work by Kearns and Martin (Ann. Rept. Long Ashton Res. Sta., 1935, 49) shows that lauryl thiocyanate is highly toxic to insect eggs and this may prove one of its most valuable uses.

Dinitro-compounds.—A new compound of considerable interest has arisen out of an active study of the dinitro-compounds as insecticides. It was found at Rothamsted that 3 : 5-dinitro-o-cresol was highly toxic to insect eggs. This material proved unsuitable for practical use for various reasons.

C. H. Richardson and J. E. Kay (J. Econ. Entom., 1936, 29, 52, 393, 397; also *ibid.*, 62, 218) have shown that if the methyl group is replaced by a cyclohexyl group to give 2 : 4-dinitro-6-cyclohexylphenol a still more powerful insecticide is obtained. It has the advantage too of being soluble in petroleum oil. It was found that this compound was toxic as a contact poison to several species of insects and their eggs and to the red spider and also that its calcium salt had stomach poison properties of a high order.

The arsenicals.—In the United States of America, in almost every insecticide laboratory the status of the arsenicals as stomach poisons is discussed. Dr. Roark and Dr. Campbell have led a brilliant team in the Bureau of Entomology and Plant Quarantine in Washington in their search for substitutes particularly for lead arsenate. It is a much more urgent problem for them than for us, as the codling moth in particular is a far more serious pest there than here, and repeated sprayings are requisite, sometimes up to a few weeks of gathering the fruit. The washing of apples before marketing in order to reduce the tolerance of both lead (0.019 grain per lb.) and arsenic (0.01 grain per lb.) to the limits imposed by the U.S.A. Food and Drug Administration became a necessity and a source of expense. It was thought that fluorine derivatives such as the silicofluorides and the fluoaluminates, which have been shown to possess powerful stomach poison properties to insects, might solve the difficulties, but the pathological changes in the teeth caused by fluorine compounds have been responsible for fixing a tolerance in America of 0.01 grain of fluorine per lb. and made washing still requisite.

The campaign therefore against lead arsenate seems to resolve itself into : (1) a search for other arsenicals not containing lead; (2) the admixture of petroleum oils with the lead arsenate; (3) the testing of low-volatile nicotine compounds; (4) the search for substitutes, chiefly organic compounds. The arsenates of calcium, manganese, and zinc, and other arsenicals seem to have given variable results. The use of oils with lead arsenate accentuates the washing problem. Nicotine-bentonite and the tannate and humate, particularly the first-named, present hopeful possibilities but have not had

a long enough trial for their exact status to be defined. Rotenone, although it can replace arsenicals for certain purposes, has proved ineffective against the codling moth. A long array of organic compounds has been subjected to laboratory trials and a few selected for further investigations. Among the most hopeful of these is thiodiphenylamine or phenothiazine,

$C_6H_4 \begin{smallmatrix} \text{NH} \\ \diagup \quad \diagdown \\ \text{S} \end{smallmatrix} C_6H_4$. This compound, synthesised by heating diphenylamine with sulphur in the presence of a catalyst, iodine or aluminium chloride, was reported by Campbell, Sullivan, Smith, and Haller to be the most toxic compound tried in insecticide tests against culicine mosquito larvae (J. Econ. Entom., 1934, 27, 1176; 1935, 28, 727; 1936, 29, 532). The material prepared on a semi-commercial scale using aluminium chloride as catalyst does not appear to have been so successful in field trials against codling moth as was expected, but it contained a good deal of inert non-toxic material. The performance, however, of the pure product in laboratory trials against codling moth causes one to look forward with interest to the results of further field application. More recently it has been reported from Washington (*ibid.*, 29, 804) that a number of azo compounds have been found highly toxic as stomach poisons in laboratory trials on mosquito larvae and the whole situation is becoming of great interest. There is, however, a gap between the laboratory and the field and a compound may stand or fall by its physical properties and its power to withstand weathering. It is probable that for such reasons lead arsenate holds its strong position rather than for its inherent toxicity.

Adjuvants.—For many years now it has been realised that if a contact or a direct-action insecticide (e.g., nicotine) in contra-distinction to a protective insecticide (e.g., lead arsenate) is to be fully effective, some other agent must be added in order to increase its wetting and spreading over the insect. Such agents often greatly accentuate toxic action. In addition, compounds are often added to enhance the adhesive properties of insecticides and to increase the stability of the emulsions used in the spray tank. An extensive literature has grown up around this phase of the subject.

A paper with many literature references by A. C. Evans and H. Martin (J. Pomology, 1935, 13, 261) gives an excellent account of the problems involved in wetting and spreading and a list of the newer materials suggested as wetters. All these materials possess this reaction in virtue of their surface activity due to a relatively massive hydrocarbon complex attached to a group polar to water, e.g., carboxyl or sulphonie or sulphate groups. Many of them have come into plant protection work via the textile trade. Although soft soap is still largely used as a wetter and spreader and is reported as being still the best for some purposes, it has grave disadvantages for use with hard water and is incompatible with some insecticides.

In this connexion attention may be drawn to an almost forgotten observation of Moore's in 1921 (Minnesota Agric. Exp. Sta. Tech. Bull. 2; J. Econ. Entom., 1925, 18, 282). Moore found by electro-osmotic experiments that a leaf, when wet, was negatively charged and that the particles of a number of arsenicals including lead arsenate when moist were also electro-negative. He found that

ferric and aluminium arsenate and arsenite were, when wet, positively charged and that lead arsenate mixed with imperfectly washed ferric and aluminium hydroxides appeared to withstand weathering and washing better than many arsenicals. The effect seems to be partly due to the presence of ferric or aluminium chloride, Hoskins and Wampler (J. Econ. Entom., 1936, 29, 134) have recently studied this phenomenon. By means of a microcataphoresis cell they determined the charges on lead arsenate and beeswax in water both with and without the presence of aluminium ions. They also studied the effect of these ions on the wetting of the beeswax surfaces and the deposit thereon of lead arsenate, when sprayed with lead arsenate-water mixtures. They found that both lead arsenate and beeswax were negatively charged when wet but that the aluminium ion induced a positive charge increasing with its concentration, but also depending on the pH value of the mixture. The change in charge was more rapid with lead arsenate than with beeswax, so that a totally unexpected increase in wetting effect took place between certain narrow limits of concentration of the aluminium ion. When sufficient aluminium chloride was added to give a low positive charge to the lead arsenate but to leave the beeswax still slightly negative the maximum deposit of lead arsenate was observed, but the deposit was greatly decreased when both were charged positively by an increase in the concentration of aluminium ions.

Mode of application

For some years now it has been suspected that the mode of application of an insecticide may have a profound effect on its toxicity. O'Kane (New Hamps. Agric. Tech. Bull., 1933, No. 54, p. 21) appears to have detected this some years ago. Some of the best examples occur in the use of pyrethrum (see Part II).

Soil fumigation

Here there are at least two grave limiting factors: (1) economic, (2) the means of evaluating results. The first may take years of further work to surmount but something has been done by attracting and concentrating the pest (e.g., wireworms) to an appropriate and easily accessible position and administering the poisons there (Ann. Appl. Biol., 1927, 14, 359; J. Econ. Entom., 1925, 18, 702). The second has been materially advanced by the recent work at Rothamsted by Ladell (Ann. Appl. Biol., 1936, 23, 862), who has applied Fisher's statistical technique to the evaluation of his field results and has elaborated an apparatus for rapidly separating the soil fauna; it operates by means of levigating process, using magnesium sulphate to bring the density of the water used above that of the insects. The insects are carried over in the foam-like scum formed by churning the soil with this solution, with the aid of a current of air. They are separated on a Buchner funnel, where they can be readily counted. The method is so successful and the numbers of insects isolated are so greatly above those separated by other methods that on comparison can be made between them.

Mode of action of insecticides

Since the early classical work of Shafer (*loc. cit.*) this aspect of the subject has been an attraction to many workers but the pressure of more urgent practical problems has only allowed, so far, intermittent investigations to be carried out. This partial neglect has been unfortunate. Shafer was aware of most of the factors involved—the importance of the surface tension of spray fluid, the relative thickness of the chitin, and the penetration through the tracheæ. He considered that vapours of insecticides chiefly found access to some vulnerable part through the tracheæ. He determined the changes in the respiratory ratio of the insect brought about by certain insecticides and states that they tend to prevent oxygen absorption by the tissues. Later he studied the effect on the enzyme system of the insect and summarised his results as follows: "Several contact insecticides, when used at a concentration sufficient to kill insects, deleteriously affected the activities of reductases, catalases, oxidases, usually in unequal degree, thus disturbing the natural or normal balance of such activities." Far-seeing too, he realised the importance of a study of the effect on nervous tissue cells but his researches unfortunately never seem to have been fully completed.

The work done since may be grouped under the following headings: (a) how the contact insecticide enters the insect; (b) the factors determining such entry; (c) the relationship between chemical constitution and insecticidal action; (d) the seat of toxic action; (e) the effect on vital processes and metabolic activities of the insect. Recent work has shown that many insecticides can penetrate directly through the insect integument (Hartzell and Wilcoxon, Contr. Boyce Thompson Inst., 1932, 4, 107; O'Kane *et al.*, New Hamps. Agric. Tech. Bull., 1933, No. 54; 1935, No. 63) and need not be taken in *via* mouthparts, spiracles, or anus, and this applies to compounds of quite varied constitution such as nicotine and white arsenic. It has been shown too that adsorption phenomena may be of great importance in determining the toxicity of a series of organic compounds to insects. In 1927 (J. Agric. Sci., 17, 183) it was pointed out by the author that adsorption was of importance in explaining the increase of toxicity with molecular weight in the case of vapours. Ferguson (Nature, 1936, 137, 361) has also pointed out that the results obtained by Price for five chloroethylenes show that the increase in the logarithms of the median lethal dose is constant and is related linearly to the boiling point. He also suggests that the effect of substituent groups in a series of substituted compounds may be purely a physical one by increasing the availability of the toxic grouping, possibly by increasing its adsorbability. True absolute toxicity, however, he believes to be determined by chemical constitution. O'Kane and his school (New Hamps. Agric. Tech. Bull., 1930, No. 39) have correlated the toxicity of nicotine, the fatty alcohols and acids with their surface activity. They have shown that the equation for the curve obtained for hexoic acid, when time to kill 50% of mosquito larvæ was plotted against concentration, was practically identical in form with the Freundlich adsorption isotherm. In the case of the median lethal doses

of a homologous series of fatty alcohols a good fit was obtained by an equation similar in form to that of Freundlich, embodying Traube's law which expresses the increasing tendency towards adsorption with increasing length of carbon chain. Bliss (Ann. Appl. Biol., 1935, **22**, 142) also considers that his log concentration probit transformation is consistent with an adsorption basis for toxicity, but that the Langmuir expression is preferable to that of Freundlich.

There is evidence to show that compounds in a molecular state are more toxic than their corresponding ions and also that they penetrate more readily. Hoskins (J. Econ. Entom., 1932, **25**, 1212) has reached the conclusion, based on the examination of the toxicity of sodium arsenite at different p_H values, that concentration at the interface, i.e., adsorption, is undoubtedly a step to penetration and that it seems more probable that toxic action takes place within the organism (e.g., by interfering with its oxidation-reduction mechanism) rather than on its surface. For optically active insecticides, e.g., nicotine, the tolylpyrrolidines, the normicotines, it has still to be shown whether the greater toxicity of one of the isomerides can be accounted for on such physical grounds. It should also be borne in mind that a serious loss of insecticidal activity may result on slight chemical change. An investigation, therefore, of the basis of chemical interaction with cell constituents,

which Gulland (Biochem. J., 1932, **26**, 32; 1935, **29**, 397) has applied to spermicidal efficiency, appears a fundamental line of attack which needs exploration in our field of enquiry. Cushny ("Biological Relations of Optically Active Isomeric Substances," 1926; Baillière, Tindall and Cox, p. 74), although dealing with a very different field of work, offers guiding suggestions of value. The following seem of importance in determining the toxicity of chemical compounds to insects: (1) the configuration of the whole molecule, the influence of which may be physical rather than chemical; (2) the presence of particular radicals the influence of which may be both chemical and physical—chemical as increasing reactivity, physical through such factors as solubility in tissue fluids, or as inducing orientation by polar groups and thus increasing adsorbability and probably penetration; (3) space arrangement, as determining the combination with some receptor. There is a good deal to learn before chemical constitution can be related with insecticidal effect, for it entails a correlation with many varied physiological reactions within the insect. Cushny believed that in his field of enquiry the methods were those of the chemist rather than of the biologist. In our field it means collaboration between the two.

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MODERN DEVELOPMENTS IN RESEARCH ON INSECTICIDES. PART II. INSECTICIDAL PLANT PRODUCTS*

By J. T. MARTIN

An account has been given in Part I of the recent work that has been carried out on insecticidal products in the inorganic and synthetic fields, together with a brief résumé of the main methods in use for the biological evaluation of such materials. There remain certain plant products, on which research is still actively in progress. Nicotine and anabasine have already been dealt with, and to these no further reference will be made. (Of the large group of plants collectively referred to as the fish-poisoning group, derris of the Far East and cubé of South America are the most important members, whilst the flowers of insecticidal pyrethrum (*Chrysanthemum cinerariæfolium*) are extensively used. The plants to which reference will be made, the localities from which they are obtained, and their native names are given in Table I.

Brazil. It is possible that in time, the Haiari plants of British Guiana, of which the white haiari is conspecific with *L. nicou*, will become of economic importance, but at present the roots in general are somewhat inferior to those of cubé. Table II demonstrates the extent of the exportation of derris from Malaya up to 1935 (Malayan Agric. Stat., 1935).

TABLE II

Exports of derris root from Malaya

Year	Tons	Value, \$
1930	90	88,176
1931	98	53,633
1932	210	92,334
1933	642	282,795
1934	602	333,520
1935	787	527,623

TABLE I

Fish-poison plants—

Derris (*Legutia*) (root)

D. elliptica
D. malaccensis
"Sumatra-type" derris

Malay States

(Tuba)

Lonchocarpus (root)

L. nicou
L. nicou
L. nicou (?)

Peru

Cubé, barbasco

British Guiana

Haiari

Brazil

Timbó

Natal

Tropical S. America

Africa

Texas

Central Africa

India

Mundulea (bark)

T. marropoda
T. toxicaria
T. vogelii
T. virginiana
M. suberosa
M. suberosa

" (leaves)

Pyrethrum—

Chrysanthemum cinerariæfolium

Japan
Dalmatia
Kenya
France

The fish-poison plants

The use of plants for the catching of fish has been known for many centuries, and the habit has been widespread in the East Indies, South America, India, and tropical Africa. It is of interest to note that the fish-poisoning plants which also possess insecticidal properties belong to the Leguminosæ. The use of *Derris elliptica* as an insecticide was first proposed by Oxley in 1848 in connexion with nutmeg cultivation. In recent years the cultivation of derris on a large scale has taken place in the East Indies, the most important centre being Malaya, whilst *Lonchocarpus nicou* is now being extensively grown for insecticidal purposes in Peru and

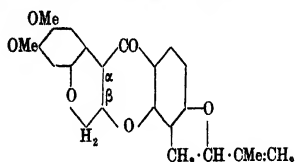
The roots are usually received in this country compressed into bales, and in such a condition, accurate sampling is rendered difficult. Samples should, therefore, be taken of the mixed, finely-ground material.

Recently Georgi (Malayan Agric. J., 1936, 24, 268) has shown that manuring increases the yield but has little effect on the toxic quality of derris root.

The chief active principle, rotenone, was first isolated in 1895 by Geoffroy from *Robinia* (*Lonchocarpus*) *nicou*, and was named by him "nicouline." Ishikawa showed that the product, termed by him "tubatoxin," was optically active, whilst Kariyone demonstrated that it contained methoxyl and could be reduced to a dihydro-derivative. The empirical formula now accepted for rotenone, $C_{22}H_{22}O_6$, was first proposed in 1928, and from

* Read before a Joint Meeting of the London Section and the Food Group on December 7, 1936.

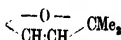
the time onwards, the work of LaForge, Haller, and Smith in America, Takei in Japan, Butenandt in Germany, and Alexander Robertson in England, led to the elucidation of the structural formula:



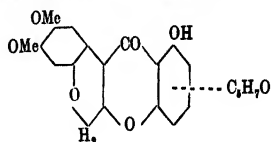
The credit for the final elucidation of the structure of rotenone rests with the American workers, although Robertson in this country arrived at the correct solution at about the same time. For the detailed account of the methods employed in the determination of the rotenone structure reference should be made to the paper by LaForge, Haller, and Smith in Chemical Reviews, 1933, 12, 181. Rotenone gives rise to a number of derivatives, of which perhaps the most important are dihydrotenone, formed by saturation of the benzofuran fraction of the molecule, and dehydrotenone, a yellow compound formed by the introduction of an oxygen atom on to the α - or β -carbon atom, followed by elimination of water. Dihydrotenone is of interest in that it is more stable than rotenone, and shows somewhat higher insecticidal activity.

It has long been known that derris extracts containing little or no rotenone may be highly insecticidal. By treating the rotenone-free derris resin in alcoholic solution with alkali, Clark (Science, 1930, 71, 396) obtained three crystalline substances allied to rotenone. These were: (1) deguelin, obtained as pale green crystals, m.p. 171°, empirical formula $C_{23}H_{22}O_8$ and therefore isomeric with rotenone; (2) toxicarol, m.p. 218–220°, empirical formula $C_{23}H_{22}O_7$, and containing one hydroxyl and two methoxyl groups; (3) a substance, m.p. 198°, and probably a constituent of the "tephrosin" isolated by Hanriot in an impure state from *Tephrosia vogelii* in 1907. Clark (*loc. cit.*) showed that the yields of these substances from derris root may be high compared with that of rotenone, and concluded that they play a part in the insecticidal action of the root. In 1930 he showed (J. Amer. Chem. Soc., 52, 2461) that both deguelin and tephrosin were constituents of the South American *Tephrosia toxicaria*, and determined their relative toxic properties to fish. Deguelin was shown to be somewhat more active than toxicarol. Clark later found (*ibid.*, 1931, 53, 313) that the deguelin, as isolated, was optically inactive, but stated that it may have occurred in the root in the optically active state.

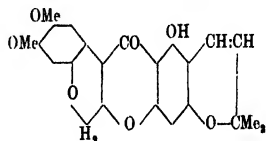
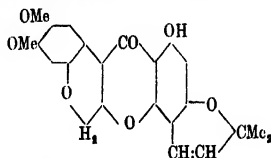
Clark proceeded with work on the constitution of deguelin, and showed its general similarity in structure to rotenone by the separation of derivatives (*ibid.*, 2007, 2369) that had been obtained earlier by LaForge, Haller, and Smith in their work on rotenone. In 1932 he showed (*ibid.*, 1932, 54, 3000) that deguelin differed from rotenone only in the benzofuran fraction of the molecule, an unsaturated pyran ring taking the place of the five-membered ring in rotenone.



Toxicarol was also shown to have a similar general structural relationship to rotenone, and in 1932 (*ibid.*, 2537) Clark suggested the following skeleton formula:



Later, Robertson (J.C.S., 1935, 681) proposed two alternatives for the structure of toxicarol:



Toxicarol is thus shown to be possibly a hydroxy-deguelin. Tephrosin and an isomeride, isotephrosin, have been isolated from the reactions involving the formation of dehydrodeguelin from deguelin. The tephrosins, which show a complicated isomerism, are hydroxy-derivatives of deguelin and are thought to be formed in the process of extraction.

Chemical evaluation of derris root

The first chemical method for the evaluation of derris was proposed in 1923 by Tattersfield and Roach (Ann. Appl. Biol., 10, 1–17), who determined the amount of material extracted by ether, and assessed its genuineness as a measure of the active principles by its methoxyl content. They found with three samples of *D. elliptica* that the amount of ether extract bore a close relationship to the toxicity of the roots to silkworms. Toxicarol, however, has approximately the same methoxyl content as rotenone, but a much lower insecticidal activity. Davidson in 1930 (J. Econ. Entom., 23, 877) tested the relative activities to *Aphis rumicis* of the isolated crystalline products. They were: rotenone:deguelin:tephrosin:toxicarol::400:40:10:1, and these ratios were obtained by Shepard and Campbell (*ibid.*, 1932, 25, 142) when the four compounds were tested as stomach poisons against silkworms. Davidson concluded that the rotenone content was the most important criterion for the evaluation of any sample, and added that, unless occurring in unusually high content, the other three constituents would not substantially affect the insecticidal quality of the root. In the light of recent knowledge this proved to be an unfortunate generalisation, in that undue prominence was given to the rotenone content of derris as a basis of evaluation.

The determination of rotenone.—In 1931, Roark (Soap, 7, No. 3, 97) outlined a method for the determination

of rotenone based on the separation of the compound from an ether solution of the derris resin. This was superseded by the method of Jones in 1933 (Ind. Eng. Chem. [Anal.], 5, 23), who extracted the powdered root material with carbon tetrachloride and concentrated the extract to a small bulk, when rotenone separated as crystals containing one molecule of solvent of crystallisation. A portion of the product was recrystallised from amyl acetate, and the identity of the product checked by its melting point.

It was found that by this method resins which had yielded no rotenone by separation from ether contained in fact appreciable amounts. Jones stated that the method was applicable to the analysis of roots containing more than 0.3% of rotenone. He determined the solubility of pure rotenone in carbon tetrachloride as 0.6 g. per 100 c.c. at 20°. It is likely, however, that the solubility of rotenone in the mother-liquor is increased by the presence of resinous material, and may lead to some loss of the rotenone complex. C. D. V. Georgi in 1933, in a publication from Malaya (Dept. Agric. S.S. & F.M.S., Sci. Ser. No. 12), utilised the separation of the carbon tetrachloride complex from the derris resin, and incorporated a recrystallisation from hot alcohol to determine the purity of the product. An allowance was made for the solubility of the rotenone in the alcohol used (0.2 g. per 100 c.c. at 20°), but recognition was again made of a possible source of loss due to the enhanced solubility of rotenone in alcohol containing non-crystallisable derris resin. Georgi prefers to call the product calculated from the rotenone-carbon tetrachloride complex "crude rotenone," reserving the term "rotenone" for the recrystallised product. In our work at Rothamsted, in view of the possibility of decomposition of the rotenone due to the high boiling temperature of carbon tetrachloride, we have used ether as extractant, with separation of the carbon tetrachloride complex from the resin, followed by recrystallisation of the complex from absolute alcohol (Ann. Appl. Biol., 1935, 22, 578).

D. R. Koolhaas (Bull. Jard. Bot. Buitenzorg, 1932, 12, 563) extracts the powdered root with ether, and, with the aid of a centrifuge, estimates the rotenone separating from the concentrated ether solution. The purity of the product is determined by its melting point, and an allowance made for occluded impurities. He states that the rotenone content should be the principal criterion in the judgment of derris roots.

Cahn and Boam in 1935 (J.S.C.I., 54, 37 r), in a critical investigation of the Jones principle for the separation of rotenone, prefer to use trichloroethylene as extractant of the dried plant material, stating that the use of this solvent considerably reduces the time of operation. In addition, the rotenone appears to be less liable to degradation when boiled with this solvent. The solvent is removed and the resin is dissolved in two parts of warm carbon tetrachloride saturated with rotenone at room temperature. After keeping overnight, the crystals are separated, washed with carbon tetrachloride saturated with rotenone, and weighed. The purification of the product is effected by trituration with five parts of alcohol saturated with rotenone.

From time to time reports have been made of the

existence of derris roots which, although toxic, yielded no rotenone by the normal method of separation of the carbon tetrachloride complex. They are further characterised by their high content of toxicarol and have been designated by Cahn (*loc. cit.*) as the "Sumatra-type" roots. He has shown, however, that rotenone may exist in such a root in a "hidden" form, and modification of his method may be made to meet this contingency. We have recently shown at Rothamsted (Ann. Appl. Biol., 1936, 23, 880) that preliminary removal of the toxicarol enables the rotenone present to separate readily from a carbon tetrachloride solution of the residual resin. Of the methods available for the determination of rotenone that of Cahn and Boam appears to be the most useful on account of its rapidity and applicability to all the economic types of derris.

In the years 1933 and 1934 further evidence was forthcoming to show that rotenone-free derris resins could still have a high insecticidal action. Takei (Ber., 1933, 66, 1826) showed the non-crystallisable fraction to be approximately as toxic to mud-fish as rotenone itself. Jones (J. Econ. Entom., 1933, 26, 451) found that a derris resin containing about 25% of rotenone was as toxic as pure rotenone when tested against mosquito larvae.

In view of these and their own observations Campbell and his co-workers (Soap, 1934, 10, No. 3, 81) again suggested the possibility of the use of the methoxyl content as a means of evaluation, in preference to the rotenone content. Speculations were made as to the possible changes which the isolated deguelin and toxicarol had undergone in the process of extraction, since, as shown earlier, these crystalline compounds showed relatively little toxicity. Clark had, indeed, referred in 1931 and 1932 (J. Amer. Chem. Soc., 53, 313; 54, 2537) to the possibility of the occurrence of these compounds in the resins in some form of combination, or in the optically active state. As isolated, they are optically inactive, whereas rotenone is levorotatory. For the isolation of the deguelin and toxicarol, the action of concentrated alkali was necessary and it was thought that this procedure may have brought about some change. Takei in 1933 (Ber., 66, 1826) reported the isolation of optically active deguelin, melting at 166° and with $[\alpha]_D^{20} = -23^\circ$, but Haller and LaForge (J. Amer. Chem. Soc., 1934, 56, 2415) considered the product to be optically inactive deguelin contaminated with a small quantity of rotenone. Haller and LaForge succeeded in preparing optically active dihydrodeguelin from a light petroleum extract of derris, and this derivative has recently been shown by Fink and Haller (J. Econ. Entom., 1936, 29, 594) to possess a higher toxicity to mosquito larvae than optically inactive dihydrodeguelin. If the toxicity of optically active deguelin exceeds that of the inactive compound to the same extent, then the active deguelin, which Haller and LaForge have shown probably to occur in derris resins, may be assumed to contribute in no small measure to the insecticidal action of the root. For further discussion on this point reference should be made to a recent paper by R. S. Cahn (J.S.C.I., 1936, 55, 259 r).

In 1935, Jones, Campbell, and Sullivan (J. Econ. Entom., 28, 255) determined the toxicity to house flies

of extracts of derris, cubé, haiari, and tephrosia roots, and endeavoured to correlate the effects observed with the results of chemical examination. The amounts of rotenone present were insufficient to account for the ethal effects, whilst the determination of the combined rotenone and deguelin expressed the relative activities of some of the roots, but not all. The methoxyl contents of the resins, after corrections had been made for the amounts of toxicarol present, gave better assessments. Recently Hoyer and Leonard (Soap, 1936, 12, No. 3, 109) have recognised the part played by the noncrystallisable constituents in their suggestion that an "index of relative toxicity" be used, whilst Jones and Smith (*ibid.*, No. 6, 113) have suggested that a definite proportion of the non-rotenone fraction of the resin be taken in conjunction with the rotenone content, as an assessment of toxicity.*

In work at Rothamsted (Ann. Appl. Biol., 1935, 22, 78) on the chemical evaluation of rotenone-bearing plants, we first compared, chemically and biologically, authenticated roots of *Derris elliptica* and *D. malaccensis*. When two samples of the same species of derris were compared, the determination of rotenone, ether extract or methoxyl content gave fairly good indications of their relative activities of *Aphis rumicis*. When, however, a sample of *D. elliptica* was compared with a *D. malaccensis*, widely discrepant assessments resulted, the *D. malaccensis* roots being more toxic than their rotenone contents alone would have led us to expect. When estimates were made, by the separation of the dehydro-derivatives, of the combined rotenone and deguelin present, much better assessments of the relative activities of the roots were obtained.

In later work (*ibid.*, 1936, 23, 880) a specimen of the "Sumatra-type" root of Cahn and Boam was incorporated in our tests. It was characterised by the inability of the rotenone to separate directly from a carbon tetrachloride solution of its resin, and by its high toxicarol content. The "Sumatra-type" root was compared in turn with a specimen of *D. malaccensis* and a *D. elliptica*. The chemical characteristics of the three types are given in Table III.

TABLE III

	% root		
	Sumatra-type	<i>D. malaccensis</i>	<i>D. elliptica</i>
Total ether extract	18.3	18.6	17.5
Rotenone	0.5	1.8	4.0
Rotenone plus deguelin*	3.1	8.2	14.9
Toxicarol*	11.9	7.3	1.1

* Based on methoxyl contents of fractions.

The roots contained approximately the same amounts of resin, but the proportion of rotenone to total ether extract was greater in the *D. elliptica* than in the *D. malaccensis*, and in comparisons of authenticated specimens of these two species we have found this usually to be the case

The large amount of toxicarol in the "Sumatra-type"

* Since this paper was sent to the press, Worsley has published details of methods for the determination of rotenone (J.S.C.I., 1936, 55, 349 r) and for the evaluation of plants containing rotenone (*ibid.*, 1937, 56, 16 r).

root invalidated the determination of the dehydro-compounds as a means of evaluating all three roots. When, however, the toxicarol was removed from the Sumatra and *D. malaccensis* roots, the estimates obtained of the rotenone and deguelin undervalued the toxicities. It was clear that the toxicarol, as it existed in the resin, was playing some part in the toxic make-up of the roots. If the concentrations used in the biological tests were expressed on a basis of rotenone plus deguelin plus approximately one tenth of the content of toxicarol, the toxicity lines (probits plotted against the logarithms of the concentrations) were almost coincident. More work is needed, however, on the exact parts played by the naturally occurring toxicarol and deguelin.

We later succeeded in isolating a crystalline, optically active compound from which a high yield of optically inactive toxicarol could be obtained by treatment of an alcoholic solution with alkali. This substance showed an insecticidal action to *Aphis rumicis* approximately one tenth that of rotenone, a figure agreeing well with the empirical value taken in determining the probit-log concentration regression lines. Inactive toxicarol derived from it showed little or no toxicity. When the substance was dissolved in benzene, it was found to have a levorotation of $[\alpha]_D^{25} -65^\circ$. If a small amount of caustic potash in methyl alcohol was added, the sign of rotation changed instantaneously. The dextrorotation then declined with time. The rate of decline was found to be less dependent on the amount of alkali added than on the amount of alcohol present. This effect was seen in similar treatment of the "Sumatra" and *D. malaccensis* resins, containing toxicarol, but was not obtained with the resin of *D. elliptica*, which contained but little toxicarol. Such an effect may have resulted in the earlier separation of the non-toxic, optically inactive derivatives of derris resins.

Buckley, in Malaya, has also utilised the separation of toxicarol from ether solutions in work on "Sumatra-type" derris, *D. malaccensis*, and *D. elliptica*, and concludes that the different varieties of derris may roughly be differentiated by their rotenone and ether extract contents and by the response of ether solutions of their resins to alkali treatment (J.S.C.I., 1936, 55, 285 r).

In our work, we have not been able to find a correlation between optical rotation of the resins and their biological activity. The rotations placed the roots in their correct toxic order, but the relationship was not quantitative (Ann. Appl. Biol., 1936, 23, 899). While much progress has been made, the possibility of the chemical evaluation of derris on a sound basis is dependent on more exact knowledge of the forms in which the toxic principles occur in the root and of their activities relative to that of rotenone.

The loss of activity of derris.—We have shown (*ibid.*, 880) that if powdered derris is stored for some years in tins under dry, cool conditions, no loss of rotenone results. On exposure to sun and ultra-violet light, rotenone gives rise to yellow derivatives, of much reduced activity. Jones and his co-workers (J. Econ. Entom., 1933, 26, 451) conclude that a thin layer of rotenone exposed to light for 10 days would probably lose its activity completely, and this fact is likely to limit the use of rotenone as a stomach poison.

Although some insects are immune to its effects, derris has a large range of applicability as an insecticide. It need only be mentioned here that the root has been usefully employed by Steer at East Malling against the raspberry beetle, whilst its success in the control of the warble fly has culminated in a recent order by the Ministry of Agriculture (J. Min. Agric., 1936, 42, 1185), by which farmers are required to dress their cattle each spring.

In recent physiological work, Tischler (J. Econ. Entom., 1935, 28, 215) concludes that derris acts primarily by deranging the respiratory functions of insects in such a way that oxygen utilisation by the tissue cells is greatly inhibited.

In South America, species of *Lonchocarpus* have in recent years been found to be powerful insecticides and to contain rotenone. The first of these to be discovered to have insecticidal properties were the haiari plants of British Guiana, shortly to be followed by cubé from Peru and timbó from Brazil. Cubé (*L. nicou*) had long been used as a fish poison. Since it is rich in rotenone, some specimens ranging as high as 11%, the cultivation of this plant for export as an insecticide is being encouraged and an embargo has been placed on the exportation of living specimens. *L. urucu* is also found in Peru, but is reported as not being so valuable. Timbó, a darker coloured root, is also coming on to the world's markets and isolated specimens rich in rotenone have been reported, but, as with derris, variations in quality are found. Cubé and timbó are likely to prove active competitors of derris root, and are being exported in increasing quantities, particularly to California and certain European markets. Nevertheless, the authorities in Malaya and Java have not been idle and by scientific plant selection, varieties of derris have been produced containing very high quantities of both rotenone and ether extractives. The prospects therefore are that we shall see a steady improvement in quality of all these roots.

Tephrosia and Mundulea species.—From *Tephrosia macropoda* of Natal we have recently separated 0.3–0.4% of rotenone, while other insecticidal principles are undoubtedly present. We have found (Ann. Appl. Biol., 1926, 13, 424) the roots of *Tephrosia toxicaria* from South America and the leaves and seeds of *T. vogelii* to be toxic to insects (*ibid.*, 1925, 12, 61). A plant recently sent to us from the British Solomon Islands, and thought to be a species of derris, also possessed leaves with insecticidal activity. Rotenone has been found in small amounts in two other species of *Tephrosia* (*virginiana* and *latidens*) occurring in the United States.

Mundulea suberosa.—Worsley (*ibid.*, 1936, 23, 311) has recently found the bark of *M. suberosa* in East Africa to be as toxic to insects as locally grown derris root containing 5.4% of rotenone. The seeds were also active but to a less extent. Tattersfield and Gimmingham in 1932 (*ibid.*, 19, 253) reported the stems, seeds, and pods of a specimen of *M. suberosa* from India to be toxic, whilst recently a plant of the same species, received by us from India, possessed leaves with appreciable insecticidal action.

The activities of these lesser known members of the fish-poisoning group may be enhanced by selection and

by other means, but with the possible exception of the East African *Mundulea*, they can hardly be said to warrant cultivation for export purposes, but may prove of value for the control of local insect pests.

Pyrethrum (Chrysanthemum cinerariaefolium)

The use of pyrethrum flowers for insecticidal purposes originated in Persia and was introduced into Europe early in the 19th century. About 1840, *C. cinerariaefolium* was first produced in Dalmatia, and superseded the less active Persian species, *C. roseum* and *C. carneum*. Later, in 1896, Japan began the extensive production of pyrethrum flowers and, during the world war, secured a monopoly which she still retains. The flowers have recently been cultivated on a large scale in Kenya Colony.

The extent of the recent production of the flowers in Yugoslavia, Japan, and Kenya is given in Table IV.

TABLE IV
Production, in tons, of pyrethrum flowers

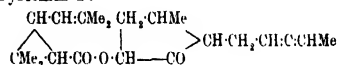
	Yugoslavia	Japan	Kenya
1930	1090	7156	—
1931	606	5560	—
1932	524	6000	—
1933	798	6400	—
1934	754	7930	41
1935	—	12,748	253
1936 up to Sept. 30th	—	—	673

It is thought that the production in Kenya for the whole of 1936 will be in the region of 1000 tons.

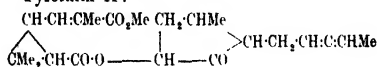
Hoyer in June, 1936 (J. Econ. Entom., 29, 605), reported the Kenya flowers to be richer than those from Japan or Dalmatia.

The active principles of pyrethrum.—The researches of Staudinger and Ruzicka published in 1924 (Helv. Chim. Acta, 7, 177) resulted in the separation and determination of the structure of two toxic substances, termed by them pyrethrins I and II. They are esters of a cyclic ketone, pyrethrolone, and two acids, termed chrysanthemum-mono- and -di-carboxylic acids. In pyrethrin II, the second acid grouping is methylated.

Pyrethrin I:



Pyrethrin II:



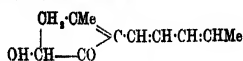
Pyrethrolone is a colourless lavoratory oil insoluble in water, but miscible with alcohol, ether, and benzene, b.p. 111–112° at 0.05 mm.

Chrysanthemum-monocarboxylic acid is a colourless liquid, boiling at 135° at 12 mm., insoluble in water, soluble in light petroleum, and volatile in steam.

The second acid, the dicarboxylic acid fraction of the pyrethrin II molecule, is a solid, m.p. 164°, with empirical formula $\text{C}_{10}\text{H}_{14}\text{O}_4$. It is non-volatile in steam.

Recently LaForge and Haller (J. Amer. Chem. Soc., 1936, 58, 1061, 1777) have re-opened the question of the nature of the side-chain in pyrethrolone. They find that

pyrethrolone may be converted into a compound identical with dihydrojasnone, and ascribe to pyrethrolone the structure:



The mixed pyrethrins may be isolated in the pure state by physical means, Wilcoxon and Hartzell (Contr. Boyce Thompson Inst., 1933, 5, 115) employing repeated extractions with light petroleum and methyl alcohol. Ripert (Compt. rend., 1935, 200, 2219; Ann. Falsif., 1936, 29, 132) has separated them by a fractional crystallisation, at low temperatures, from light petroleum solution. La Forge and Haller (J. Amer. Chem. Soc., 1935, 57, 1893) have utilised fractionation between light petroleum and acetic acid in the separation of pure pyrethrin II.

The determination of the active principles.—Many methods have been devised depending on the reducing action of the pyrethrolone, or on the determination of the pyrethrin acids. The most widely used reduction method is that of Gnädinger (*ibid.*, 1929, 51, 3054), who determines the reduction of an alkaline copper solution colorimetrically. Tatu (Parfumerie moderne, 1930, 24, 607) and Raupp (Apoth.-Ztg., 1935, 75, 216) have modified the Gnädinger method and determine the extent of reduction volumetrically. Staudinger and Harder (Ann. Acad. Sci. Fennicæ, 1927, A, 29, No. 18, 1) estimated the pyrethrins by the determination of the acids, but their method requires a large amount of material. It was modified by Tattersfield in 1929 (J. Agric. Sci., 19, 266) and revised in 1931 (*ibid.*, 21, 115). We have shown (*ibid.*, 1931, 21, 115) that over a wide range of samples, the results by the Gnädinger and Tattersfield methods show good agreement, and this has been confirmed by Hartzell and Wilcoxon (Contr. Boyce Thompson Inst., 1932, 4, 107). Seil (Soap, 1934, 10, No. 5, 89; Chem. Trade J., 1934, 95, 168) has devised a modification of the Tattersfield method more suitable to works practice, but Gnädinger (Soap, 1934, 10, No. 9, 89) finds that the Seil method tends to give slightly higher figures for the total pyrethrins than his own.

Ripert (Ann. Falsif., 1934, 27, 580; 1935, 28, 27) determines the total pyrethrin acids, separates the volatile acid, and obtains the pyrethrin II acid by difference. Haller and Acree (Ind. Eng. Chem. [Anal.], 1935, 7, 343) have proposed an alternative method for pyrethrin II based on the determination of the methoxyl group. They state that, in general, the results for pyrethrin II are lower than those by the Tattersfield or Seil methods.

More recently, Wilcoxon (Contr. Boyce Thompson Inst., 1936, 8, 175) has published a further method for pyrethrin I, based on the action of the monocarboxylic acid on Denigès' reagent.

Gnädinger and Cori (J. Amer. Chem. Soc., 1930, 52, 3300) show that the determinations of the total pyrethrins by their method will assess the toxicity of the flowers, whilst Tattersfield (J. Agric. Sci., 1929, 19, 266) and Richardson (J. Econ. Entom., 1931, 24, 1098) have indicated that the determination of pyrethrin I may be sufficient for this purpose. In addition, both Tattersfield and Gnädinger have shown that their methods will detect deterioration of the flowers on exposure or storage.

Tattersfield in 1931 (Ann. Appl. Biol., 18, 602) traced the development of the poisons as the flowers matured, and found a maximum content when the heads were fully open; this has also been shown by other workers. The belief that the immature flowers were the most valuable was previously held. The poisons were found to be located to a great extent in the ovaries of the flowers. It was also shown that the poison content could not be influenced by manuring, whilst other experiments at Rothamsted showed the necessity of a dormant period for the normal flowering of the plant (*ibid.*, 1934, 21, 670, 682).

The relative toxicities of pyrethrin I and II.—Staudinger and Ruzicka considered pyrethrin I to be more toxic than pyrethrin II to roaches, whilst Tattersfield and his associates (J. Agric. Sci., 1929, 19, 266) found pyrethrin I to be approximately ten times as toxic as II to *Aphis rumicis*. Gnädinger and Cori (J. Amer. Chem. Soc., 1929, 51, 3054; 1930, 52, 3300), in work on roaches, found pyrethrin I to be slightly more active than II, whilst Wilcoxon and Hartzell (Contr. Boyce Thompson Inst., 1933, 5, 115), working again with aphids, supported Tattersfield's contention that pyrethrin I was much more active than II. Ripert and Gaudin (Compt. rend., 1935, 200, 2219; Ann. Falsif., 1936, 29, 132) show that on injection into frogs, pyrethrin II is slightly more toxic than I, but that against insects pyrethrin I has the greater activity. The position has been clarified by the recent work of Hartzell and Wilcoxon (Contr. Boyce Thompson Inst., 1936, 8, 183) using extracts rich in pyrethrin I or II on *Aphis rumicis* and house flies. They find that the relative activities of the two compounds may be influenced by the mode of application. When the pyrethrin II was applied in true solution in a miscible oil (penetrol), its activity approached that of pyrethrin I applied in the same way. When used in aqueous sprays, with the pyrethrins thrown out of solution, the activity of the pyrethrin II was considerably lower than that of the pyrethrin I. The physical condition of the pyrethrins at the time of application may thus be a determining factor in the relative activities found.

Loss of activity of pyrethrum.—It has been known for many years that on storage the flowers gradually lose their activity. Tattersfield (J. Agric. Sci., 1932, 22, 396, 1934, 24, 598) found that if an extract of the flowers, spread on talc or other finely divided inert material, was exposed to sun or artificial light, the activity was rapidly lost. Both light and air were necessary for the inactivation, since pyrethrum-talc preparations exposed to air in the dark, or to nitrogen in sunlight, lost their activities much less readily. The incorporation of certain antioxidants such as pyrocatechol, resorcinol, quinol, pyrogallol, and tannic acid conferred a measure of protection against inactivation. Alcoholic and petroleum extracts were found to be more stable than had generally been supposed (Ann. Appl. Biol., 1931, 18, 203). Gnädinger (Ind. Eng. Chem., 1932, 24, 901) also found a loss in activity of 30% when ground flowers were stored in closed containers for one year. Ripert, however (Ann. Falsif., 1934, 27, 580; 1935, 28, 27), believes the loss of activity to be due not so much to the decomposition of the pyrethrins, but to the mechanical coating of the

active principles by oxidised acids, rendering them insoluble in light petroleum. Gnadinger believes that the pyrethrins, on inactivation, form resinous compounds insoluble in light petroleum, thus accounting for the ability of the analytical methods to detect deterioration.

In the commercial manufacture of extracts, ethylene dichloride has been found to be the most useful solvent, whilst antioxidants (e.g., substituted *o*-dihydroxy-benzenes, aminoanthraquinones) are incorporated for their stabilisation.

Pyrethrum flowers are used to a large extent in household sprays, particularly in America, it being customary now to incorporate a derris extract as well. Large quantities are used in the preparation of livestock sprays (e.g., control of stable fly) and as horticultural dusts and sprays. In Germany, forest pests have been treated by means of pyrethrum-talc dusts applied by means of aeroplanes, and this method of distribution of pyrethrum dusts on a large scale has been used in the United States.

The mode of application of insecticides may have a profound effect on their toxicities, as seen in the work of Hartzell on the pyrethrins. In Kenya, LePelley (Bull. Entom. Res., 1933, 24, 1) has secured satisfactory control of *Antestia* and *Lygus* pests on coffee by the use of atomised paraffin extracts of pyrethrum, but found aqueous emulsions to be of little value.

Potter has recently shown (Ann. Appl. Biol., 1935, 22, 769) that a good control of the warehouse pests *Ephestia elutella* and *Plodia interpunctella* may be achieved by the atomisation of a pyrethrum extract in white oil. Under such treatment, food warehouses which a few years ago were heavily infested have been largely freed from the pests.

Action of the pyrethrins on the insect.—In contrast to the action of rotenone, which brings about a gradual paralysis of the insect, the action of pyrethrum is rapid. Hartzell and Wilcoxon (Contr. Boyce Thompson Inst., 1932, 4, 107; 1933, 5, 115) found that pyrethrin preparations placed on the integument of insects removed from the spiracles or other body openings, caused death. The penetration of the integument was observed by adding a dye to the concentrate. It is believed that the poisons are then carried to the nerve ganglia in solution in the body fluid. Death was found to be due to the destruction of the cells of the central nervous system, accompanied by paralysis. Insects killed by administration of rotenone did not show the typical nerve lesions produced by pyrethrum.

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THE ARTIFICIAL DRYING OF PYRETHRUM FLOWERS

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INTRODUCTION.

With the more extensive cultivation of Pyrethrum, *Chrysanthemum cinerariaefolium* in recent years, the problem of drying the flowers to such a condition that they are suitable for transport and storage, has assumed considerable importance. A large proportion of the flowers used for the purpose of preparing insecticidal extracts or powders, has formerly been dried to such a condition merely by exposure to the air, in thin layers, or, under favourable conditions, to direct sun. Much space and labour is necessary to deal with flowers in this way and the method can hardly be regarded as satisfactory except where only small quantities are handled or where very favourable weather conditions prevail. Moreover, it is not certain that such methods of drying result in the retention of the maximum pyrethrin content for, in the case of hops, it appears that when they are dried at room temperature, the α resin content is inferior to that obtained when comparable samples are dried artificially (Burgess 1931). From the point of view alone, however, of handling a large bulk of flowers, there are obvious advantages to be gained from some form of artificial drying of Pyrethrum and the question at once arises as to what temperature may safely be employed in such a process.

For some years past, an half acre plot of Pyrethrum has been grown at Wye for the purpose of making observations on the cultivation and harvesting of the crop (Jary 1936). Crops of up to 22 cwt. of fresh flowers have been obtained from this plot and throughout the period of seven years during which it was continued, the whole bulk of flowers was dried on a hop kiln. This proved very suitable for the purpose but it was not possible to carry out critical experiments relating to the optimum drying temperature. Originally a kiln of the "open fire" type was used, in which drying was done by means of an air current, from a fire on an open hearth, ascending through a layer of flowers on the drying floor above. In 1934 a newer type of kiln became available, which employs a forced draught of air. This is produced by a large fan and led through steam-heated radiator blocks before passing up to the drying floor. Whereas in the older type of kiln the temperature was controllable only within very wide limits, in the newer type it could be maintained accurately within a few degrees of any desired point. The new kiln was however far too costly to operate for experimental samples, but a smaller one, operating on a similar principal and used for experimental work on hops became available. This kiln was designed by Mr. A. H. Burgess of the Hop Research Department, South-Eastern Agricultural College, and we are greatly indebted to him for placing it at our disposal. A scale drawing and brief description of this kiln are given below (p. 110).

OBJECT AND SCOPE OF EXPERIMENT.

In drying flowers on the larger kilns, some arbitrary temperature had to be aimed at and there was little evidence to show what was best in this respect. Consequently the kiln was operated at a temperature approximately the same as the maximum employed in hop drying, 140° F. to 150° F. Judging from the pyrethrin content of flowers dried under these temperature conditions (Jary 1936) very little if any reduction seems to have been brought about, though no air-dried controls were retained to check this. It was decided, however, when the small experimental kiln became available, to dry comparable samples over a range of temperatures and the points 45° C. (113° F.); 52° C. (126° F.); 60° C. (140° F.); 68° C. (154° F.) and 75° C. (167° F.) were fixed for the purpose.

HARVESTING OF SAMPLES.

It has been shown (Gnadinger, 1936; Ripert, 1935; and Tattersfield, 1931) that the pyrethrin content of flowers reaches a maximum at a period immediately preceding the formation of seed. After fertilization, the weight of the flower head rapidly increases without any corresponding increase in the amount of pyrethrins so that, though the actual amount of pyrethrins remains approximately the same, the percentage by weight rapidly falls as seed-formation progresses. It is therefore generally accepted that the optimum time for harvesting is reached when the flowers are fully open but the formation of seed has not proceeded to an appreciable extent, this stage being attained when about one-half to two-thirds of the disc florets are open.

When plants are grown from seed taken at random from a number of parent plants, it is always found that the progeny vary considerably in type, size and weight of flower, pyrethrin content and the time at which the bulk of flowers are open. In such a population, flowers continue to come to maturity over several days and there is normally a period of about ten days following the opening of the first flowers before any large proportion of them become "overblown". The plots from which flowers were gathered for the experiment here described, possessed a miscellaneous population of this kind and were a series of six plots raised from seed selected from six parents (Jary, 1936). It was known that roughly two-thirds of the plants in each plot conformed to one parent type, the remainder resembling those of the other five. As there was one plot of each type, the whole six, considered as a single unit, contained about the same number of plants of each. In order to overcome errors due to the slightly earlier flowering of any one type, picking was not commenced until all the plants were well in flower and then any "overblown" flowers were discarded. When picking the samples, only those flowers in the optimum stage of development were taken. Each flower was picked individually by hand and approximately the same quantity was taken from each of the six plots at every picking. That such differences as might have existed in the pyrethrin content of the various types of flowers were overcome by the precautions, is evident from the figures shown in Table II for the air-dried samples.

SAMPLING.

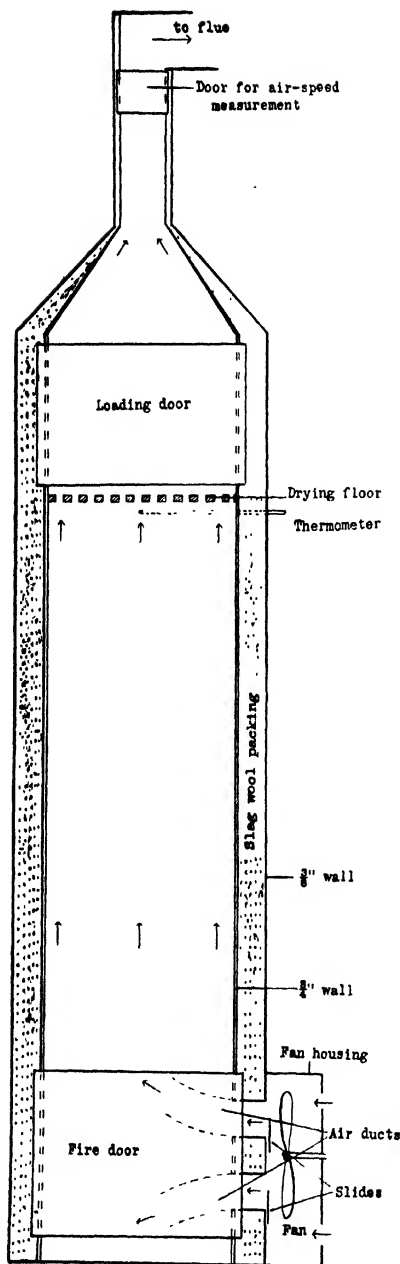
About 20 lb. of fresh flowers were picked for each drying test. They were spread out thinly on a large sheet, very thoroughly mixed together by hand and finally quartered. Two separate quarters were taken at random, one-half of the flowers being placed immediately on the kiln and the other spread out to air-dry.

THE EXPERIMENTAL KILN.

Fig. 1 represents a diagrammatic front view of the kiln used, drawn to the scale of $\frac{1}{2}$ in. to 1 foot. It is of square section, with an internal measurement of 2 ft. by 2 ft. The walls are composed of a hard asbestos composition board, the inner wall being about $\frac{1}{4}$ in. thick and the outer about $\frac{3}{8}$ in. thick. The space between the walls is packed with slag wool to minimise loss of heat. The whole kiln stands on a concrete base and the flue at the top is built into the side of a chimney. The small uppermost door is situated in a part of the flue which has a cross section of one-quarter of a square foot so that, if the linear rate of air flow be measured and the time is known, the total volume of air passing through the kiln can be calculated readily. The upper door in the main body of the kiln admits of loading and unloading flowers, the slatted drying floor being situated immediately below it. A thermometer occupies a position just beneath the drying floor. The lowermost door gives access to the fire, in this case, a series of gas burners, and two air ducts enter at this level. These connect with the housing of an electric fan bringing in air at room temperature and the opening of each air duct may be regulated by means of a slide.

KILN-DRYING TECHNIQUE.

The drying floor of the kiln was covered with a layer of cheesecloth and on this the flowers were placed to an even depth of about $1\frac{1}{2}$ in. No preliminary drying of the flowers had taken place and the kiln drying was commenced within half-an-hour of picking. The kiln had previously been regulated to work at the required temperature and the fan set to give the necessary air-speed. Immediately the flowers were spread, the door was closed and not reopened for a few hours, when a few flowers from various positions in the layer were examined. When it was estimated that about another hour would complete the drying, the flowers were turned by hand to ensure even drying but this was probably



Scale:- $\frac{1}{2}$ " = 1'

not necessary in so thin a layer. As a criterion of the final dry condition, the ease with which flowers broke under slight pressure was taken, the following method being employed for determining this point: a flower with the disc uppermost was held between the thumbs and forefingers of both hands and a slight outward and downward pressure exerted with the thumbs. In dry flowers the achenes readily broke away at the bases, but, if still not dry, they bent over and did not separate. Drying was carried on until, in a sample of flowers drawn from various parts of the layer, all responded to this test. The flowers were then at once removed from the kiln, allowed to cool for a few minutes and then packed in stout paper bags, the tops of which were loosely folded.

The temperature of the kiln was maintained by means of a number of gas rings and burners placed on the floor inside the bottom door and the number employed varied with the temperature required. Thermostatic control was not fitted to the kiln, but the temperature was regulated without much difficulty by adjustment of screw clips on the rubber gas leads. Once set, only slight and gradual fluctuation of temperature occurred and by occasional reference to the thermometer, this was quickly observed and rectified. In practice, the temperature did not vary more than 2° C. from the required point.

The dates of harvesting, the temperatures of the kiln, periods required for drying and the rate of air flow through the kiln, are shown in Table II.

AIR-DRYING TECHNIQUE.

As soon as a sample of flowers was placed on the kiln, the corresponding control sample was laid out to dry at air temperature. A long storage room was used for this purpose, through which a slow air current was maintained by means of a window at each

TABLE I.

Maximum and Minimum Temperatures for room in which samples were air-dried.

Date.	Max. °F.	Min. °F.
June 24	78	—
25	77	—
26	77	64
27	66	60
28	72	61
29	77	64
30	68	62
July 1	69	62
2	69	61
3	63	60
4	66	62
5	77	65
6	77	64
7	78	66
8	74	59
9	70	62
10	63	56
11	64	56
12	68	57
13	64	60
14	69	60
15	68	60
16	65	59

end. No direct sunlight fell upon the flowers. Each sample of flowers was placed on a flannel sheet, supported upon a layer of wire netting raised about 18 in. above the floor so that there was good ventilation all round the flowers. In all the air-dried samples the flowers were placed in a single layer only and they were arranged along the room so that all were subjected to comparable drying conditions. When finally dry, the flowers were packed into bags similar to those used for the kiln-dried samples. The maximum and minimum temperatures recorded in the room for the period of drying are shown in Table I.

ANALYTICAL RESULTS.

The analyses were carried out during August 1936, the flowers being quartered and one-half finely ground immediately before analysis and placed in tins with lever lids. There was little or no possibility of their deterioration on storage before analysis. The Seil (1934), Wilcoxon (1936) and Haller (1935) methods were employed. In the two first-named methods, the free acids were removed by extraction of the petroleum ether solutions with 0.1 N. aqueous potash. In the Wilcoxon method, the precise limits given by him for carrying out the method were not exactly complied with, as the samples were richer in pyrethrins than expected. The figures obtained by this method are not given in Table II for this reason, but in each case the pyrethrin 1 content was appreciably higher than that shown by the Seil method. The relative differences between the kiln-dried Pyrethrum and its air-dried control for each test, were, however, of the same order. The figures for the Haller method are given in Table II as they confirm previous findings for the kiln-drying effect on the apparent pyrethrin 2 content as determined by this method. The Haller technique shows an apparent increase in pyrethrin 2 content in the kiln-dried samples over the air-dried samples for all temperatures used. We are unable to correlate this effect with the temperatures of the kiln

TABLE II.

Analytical results from Pyrethrum flowers dried at different temperatures.

Date.	Method of drying and temperature of kiln.	Time (hrs)	Moisture content %	On dry matter basis.		
				Seil method.		Haller method Pyrethrin 2 %
				Pyrethrin 1 %	Pyrethrin 2 %	
June 26	Air-dried	—	7.8	0.75	0.79	0.69
June 26	45° C. (113° F.)	21	7.5	0.71	0.72	0.84
June 30	Air-dried	—	9.6	0.80	0.74	0.67
June 30	52° C. (126° F.)	10	8.6	0.78	0.74	0.82
June 24	Air-dried	—	8.1	0.70	0.76	0.72
June 24	60° C. (140° F.)	6½	8.6	0.74	0.75	0.89
July 2	Air-dried	—	7.9	0.86	0.79	0.74
July 2	68° C. (154° F.)	5½	8.1	0.75	0.63	0.78
June 28	Air-dried	—	7.7	0.76	0.75	0.67
June 28	75° C. (167° F.)	3½	8.0	0.68	0.67	0.80

All the samples were of good colour.

Air speed through kiln 36.6 linear feet per minute in all cases.

or the time taken in the process of drying. Also, when the pyrethrin 2 contents, as determined in the air-dried samples by the Haller and Seil methods are compared, it is found that the Haller method gives somewhat lower results, whereas for the kiln-dried samples the figures are uniformly higher. We can only conclude that the Haller method is invalidated for the determination of pyrethrin 2 in kiln-dried samples but we are unable to offer any explanation of this curious effect. It is, however, very unlikely that it is due to the absorption of products of combustion of the coal-gas used in the drying process.

DISCUSSION OF RESULTS.

In discussing the results given in Table II, it is necessary to point out that a certain allowance has to be made for sampling error. Under the conditions of the experiment, this could not be estimated, but small differences in the determination of the pyrethrins may be due to this cause rather than to the method of drying and, in the case of the kiln-dried samples, where the pyrethrin 2 content does not fall *pari passu* with the pyrethrin 1 content when compared with the air-dried control, we have regarded sampling error as the responsible factor. Thus, for the material kiln-dried at 52° C. (126° F.), there is a small loss of pyrethrin 1 but none of pyrethrin 2 and this is ascribed to sampling error as is the rise in pyrethrin 1 content for the sample kiln-dried at 60° C. (140° F.).

The results in Table II indicate two effects, one due to the temperature and the other to the time of drying. In the sample kiln-dried at 45° C. (113° F.), 21 hours was taken in the process. There is a slight loss of both pyrethrins 1 and 2, which is probably significant. Such loss can hardly be ascribed to the temperature of the kiln, apart from the time taken in the process of drying, for samples dried at higher temperatures, 52° C. (126° F.) and 60° C. (140° F.), for shorter times, do not show the same degree of loss. There is a distinct loss of pyrethrins in the samples dried at 68° C. (154° F.) and 75° C. (167° F.) and, as the time of drying was relatively short, the loss in these cases must be ascribed to the temperature used. The samples dried at 52° C. (126° F.) and 60° C. (140° F.) are not significantly different in pyrethrin content from their respective air-dried controls and we consider that temperatures of this order may be regarded as comparatively safe for drying *Pyrethrum* flowers by the kiln method, but the data show that temperatures above 60° C. (140° F.), may possibly result in loss of activity.

Beckley (1936) and Beckley and McNaughton (1937) have published reports on their experiments in drying *Pyrethrum* flowers in Kenya. As far as can be judged from the data presented, their conclusions do not differ greatly from those we would draw from our experiments, except that, in our case, we did not find flowers off-coloured as a result of kiln-drying.

Beckley states that there is no appreciable loss, if any, in pyrethrin content, when drying is carried out at 50° C. (122° F.). The loss is small but appreciable at 60° C. (140° F.) and increases with rise of temperature. He recommends that an effective temperature of 54° C. (129° F.) should not be exceeded. Our experiments would indicate that this temperature, 54° C.-55° C. (129° F.-131° F.) is near an optimum for purposes of drying *Pyrethrum* flowers and if it errs at all does so on the safe side.

Beckley also points out that rapid drying in the early stages may prolong the process and give rise to an unevenly dried product, through the case-hardening of the achenes. He suggests that it would be better to commence drying the flowers at a temperature not above 40° C. (104° F.) and that when the flowers have wilted the temperature may

be raised to 60° C. (140° F.). It is of interest to note that a very similar practice is followed in drying hops in England, but for other reasons (Burgess, 1933). The difficulty observed by Beckley was not met with in our experiments, but it is impossible to say, from his published data, in what way his drying process may have differed from ours. It should be borne in mind that the initial evaporation of water from wet flowers would lower the temperature of the air in immediate contact with them. In addition, our experiment was carried out on a relatively small scale and if drying is done on a larger scale, the possibility of too high an initial temperature giving rise to uneven drying should be taken into account.

SUMMARY.

An experiment is described on the artificial drying of *Pyrethrum* flowers at different temperatures. The apparatus used is described and the pyrethrin content of the kiln-dried flowers given for comparison with their air-dried controls.

There is a loss of pyrethrins in the sample dried at 45° C. (113° F.) for 21 hours and in those dried at 68° C. (154° F.) and 75° C. (167° F.) for 5½ and 3½ hours respectively. There is little or no loss of pyrethrins in samples dried at temperatures of 52° C. (126° F.) and 60° C. (140° F.) when comparisons are made with their air-dried controls.

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FIELD EXPERIMENTS ON THE CONTROL OF APHIS-TRANSMITTED VIRUS DISEASES OF *HYOSCYAMUS NIGER*

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(With 2 Text-figures)

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I. INTRODUCTION

IN a previous paper(2) descriptions were given of two plant viruses, *Hyoscyamus* virus II and *Hyoscyamus* virus III, obtained from Henbane (*Hyoscyamus niger*) which is grown by Messrs William Ransome and Son, Limited, Hitchin, for the manufacture of pharmaceutical preparations. Both viruses were found to be transmissible by the aphid *Myzus persicae* Sulz. They had similar physical properties and host range, but Hy. III caused a more severe disease than Hy. II. This suggested that they might be strains of the same virus. It was found later, however, that Hy. II would infect potatoes whereas Hy. III would not, and Hy. II was identified

by K. M. Smith as a strain of potato virus Y⁽³⁾. Hy. III does not appear to be closely related to either Hy. II or potato virus Y, because inoculation of a plant with either of the two less virulent viruses will not "protect" against inoculation with Hy. III.

Hyoscyamus plots were kept under observation for 2 or 3 years and it became obvious that the presence of these two viruses in some years caused serious loss in yield. Attempts to check the spread of similar viruses in other crops, e.g. potatoes, by controlling the aphid vectors are reported to have been unsuccessful, but there were some peculiarities about the cultivation of *Hyoscyamus* which suggested that such measures might be more successful on this crop.

Hyoscyamus is a biennial, and crops are taken from the same plants in 2 consecutive years. Messrs Ransome sow it as a rule in October in plots of about 5 acres. Generally two such plots are sown in each year, one at the Hitchin farm and one at Meppershall 11 miles away. The plants appear above ground in late April or May and grow in rosette form until July when a leaf crop is taken. During this period they are particularly susceptible to infestation with *Myzus persicae*. A second crop is taken from the same plants in September of the first year. New growth begins in March of the following year and a third crop is taken in May or early June. In good years, which are probably years when virus infection is low, a separate flower crop is taken. Virus infected plants flower late and have few flowers. The plants are then ploughed in and followed by cereals, sainfoin or roots. Potatoes were formerly used as a rotation crop, but this practice has been discontinued owing to danger of virus infection. The cropping was arranged so that in each of the two fields there was one plot of *Hyoscyamus* in its first year, one in its second and one under the rotation crop.

Since the time of maximum aphid infestation occurs generally in June or July (Davies⁽¹⁾) the period before the first cutting is probably of greatest importance. By the time the second growth has appeared in the first year the aphid infestation is over, and in the second year the crop is generally cut before any infection received in that year has had time to reduce the yield. On the other hand, the two cuttings in the first year serve to spread the virus mechanically so that any infection introduced in the first period is intensified in the third crop which is the heaviest in yield.

It therefore seemed practicable, and possibly economic, to increase the chance of successful insect control by concentrating control measures on the first 2 or 3 months of growth.

Attempts were also made to find out whether the effect of spraying was particularly large for any period of growth, either when the plants were unusually susceptible, or when aphid infestation was at its maximum. This might enable the spraying period to be reduced still further.

11. METHODS

With Messrs Ransome's permission and co-operation, part of the *Hyoscyamus* plots on the Meppershall farm were laid out as spraying experiments for the 2 consecutive years, 1934 and 1935. Observations were also made on the 1935 and 1936 harvests of these two experiments. The aim was to find out the effect, on virus infestation and crop yield, of spraying once weekly for the whole period preceding the first harvest and also weekly in May, June and July separately.

A knapsack sprayer was used, of 3 gall. working capacity. The 3 gall. of solution contained 20 c.c. of 95 per cent commercial nicotine and $\frac{1}{4}$ lb. green soft soap made up in rainwater. The plants were sprayed on both sides of each row and the insides of the guard rows (see below) were also sprayed. From 1 to 2 gall. were used per plot according to size of plants.

In the field it is often difficult to distinguish between the symptoms caused by Hy. II and Hy. III viruses because they vary with the length of time the plants have been infected. The only certain method of identification is to compare them in experimental plants. By these means it has been found that Hy. II is very much more common in the field than Hy. III although, as can be seen by the following figures, Hy. III is the more infectious, both by mechanical and by insect inoculations:

No. of starch lesions obtained by mechanical inoculation on opposite half-leaves of tobacco plants. Extracts from plants of same age, and given identical treatments	{ Hy. II, 6,389 Hy. III, 12,324
Percentage infection obtained by means of <i>Myzus persicae</i> in identical experiments	{ Hy. II, 16.1% Hy. III, 31.9%

As the method of inoculation of samples from the field was found to be inadequate for estimation of total infection it has not been practicable to distinguish between the two viruses in these observations.

Because of differences in the topography of the plots and in the dates of germination the design and treatments differed in the 2 years during which spraying experiments were carried out. They will therefore be discussed separately.

III. EXPERIMENT 1, 1934-5

A. ARRANGEMENT AND TREATMENTS

Meppershall farm is part of a shallow valley lying east and west. The *Hyoscyamus* plot sown in October 1933 was a rectangle of about 5 acres. It was situated on the north side of the valley so that the ground rose slightly towards the north.

Exp. 1 was laid out in the south-east corner of this *Hyoscyamus* plot and took the form of a 5 × 5 Latin square with arrangement and treatments as in Fig. 1. The portion selected for the experiment appeared to be fairly uniform as far as plant growth was concerned.

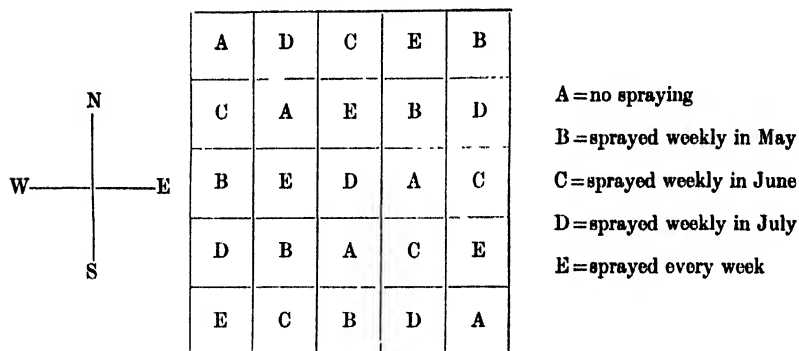


Fig. 1. Arrangement and treatments in Exp. 1.

Each experimental plot was 10 yd. long and 10 rows wide, the rows spaced 33 in. apart. Two rows, and a width of 1 yd. across the rows, were left as a guard between the plots. Without the guard rows the area covered by the experiment was roughly half an acre.

B. RESULTS

(1) *Duration of spraying*

The plants appeared above ground during the first week in May and spraying was started immediately, although no aphides were observed on the plants. Spraying ceased 1 week before the first harvest, the last spraying date being 20 July.

(2) *Observations, 1934 (first-year crop)*(a) *Aphis counts.*

Weekly observations, immediately before spraying, were made of the numbers of adults and nymphs of *Myzus persicae* on 10 plants selected

at random from each plot, 250 in all, during the last 4 weeks of spraying. The first aphides were observed on the plants on 22 June, but counts were not made until 6 July. The results of the four weekly counts made in July are given in Table I.

Table I

Counts of infested plants, adult aphides and nymphs, Exp. 1, 1934.
(Numbers per plot, i.e. 10 plants)

	Date of observations	Treatments (mean of 5 plots)					s.e.	Mean
		A	B	C	D	E		
Plants infested (out of 10)	6 July	6.2	4.6	2.2	3.2	3.0	± 0.78	3.84
	13 "	8.0	8.6	7.6	6.2	4.2	± 0.47	6.92
	20 "	8.4	7.4	8.0	6.0	5.2	± 0.42	7.00
	27 "	10.0	10.0	9.8	8.4	7.4	± 0.58	9.12
	Mean	8.07	7.31	6.90	5.95	4.95	± 0.304	6.63
No. of adult aphides (per 10 plants)	6 July	11.8	8.6	2.4	9.0	3.8	± 1.54	7.12
	13 "	23.2	23.4	9.8	8.2	6.8	± 3.49	14.28
	20 "	48.8	17.0	20.8	8.6	6.8	± 8.80	20.40
	27 "	74.8	49.8	44.0	15.4	11.2	± 7.67	39.04
	Mean	39.65	24.72	19.25	10.30	7.15	± 3.727	20.21
Nymphs (per 10 plants)	6 July	23.6	21.0	2.8	4.0	2.6	± 5.88	10.80
	13 "	71.0	80.0	50.6	44.4	33.0	± 10.26	55.80
	20 "	54.0	53.6	33.0	23.2	10.2	± 13.82	34.80
	27 "	175.2	115.4	75.4	37.4	31.4	± 23.00	80.76
	Mean	64.76	56.00	35.96	21.80	15.44	± 15.746	36.43

At each weekly count the sprayed plots gave smaller numbers of aphides and fewer plants infested than the unsprayed plots. The differences between spraying every week (E) and spraying only in July (D) and also between spraying only in June (C) and spraying in July were not significant for all occasions, but E, D and C were all significantly below the no-spray level, except in the nymph counts when only the difference between A and E was significant. The numbers found on plots sprayed only in July (B) were not significantly different from A or C but appeared to be intermediate.

(b) *Estimation of virus infection.*

On 29 July, 1 week after the last spraying date and about 3 days before cropping, an attempt was made to estimate the percentage of virus infection. Ten plants were selected at random from each plot, giving 250 samples in all. A few leaves from each were preserved in individual numbered envelopes. They were macerated separately in small mortars, the ground glass pestles of which were used to rub the extracts into the leaves of healthy tobacco plants.

Only 2.5 per cent of the test tobaccos became systemically infected, though the percentage of infection in the field had appeared to be fairly

high. It was not, however, until the following year that a possible source of error was discovered, and by this time it could not be checked by field observations. Using exactly the same technique in 1935 it was found that although most of the leaves inoculated with infected juice from the field showed local necrotic lesions 2 or 3 days before the appearance of any systemic symptoms, systemic symptoms actually appeared on only a very low percentage of the test plants. It is possible that necrotic lesions were also formed on the plants inoculated in 1935; and as local lesions were not expected from Hy. II and Hy. III inoculations their appearance had been overlooked, so that only the small proportion of systemic infections were regarded as positive. Otherwise it would have to be supposed that a very large increase in percentage infection took place between July 1934 and June 1935 when there was 58.4 per cent infection. Also the spread of virus infection during the remaining months of 1934 must have taken place within the individual plots, since there is a significant difference for treatments in the 1935 yield (see p. 563), which could not be attributed to the effect of 2.5 per cent infection.

(c) *Yield.*

No estimate of crop yield was made for the 1934 crop of Exp. 1.

(3) *Observations, 1935 (second-year crop)*

(a) *Aphis counts.*

The first counts in 1935 were made on 100 plants selected at random. Altogether 18 adults and 44 nymphs were found, so that this apparently represented the beginning of the infestation. Further counts were made on 250 plants on 18 May when the total had risen to 270 adults and 1366 nymphs. There were no differences in aphid numbers between the 1935 treatments.

(b) *Estimation of virus infection.*

Inoculations were made from 250 leaf samples collected on 12 June, the date of cropping. The following numbers of test plants were infected:

	Plants	Infected	Infection %
A. No spray	50	28	56
B. Sprayed weekly in May	50	38	76
C. " June	50	31	61
D. " July	50	19	38
E. Sprayed every week	50	30	60
Total	250	146	58.4

There were no significant differences between treatments and no indication of correlation between percentage infection and yield. This

suggests that either the method of estimating percentage infection was not sufficiently accurate, or, as aphides were present on the plants from 10 May onwards till cropping time on 12 June, uncontrolled spread of virus infection may have taken place during this period. According to the findings for Exp. 2 (see p. 568) this would not necessarily affect the yield.

(c) *Yield.*

The crop was harvested on 12 June, which was unusually late. The total yield of about 3 tons per acre was rather above the average.

Estimates were made of the yield from the experimental plots by weighing 1 yd. lengths selected at random from each row of each plot, as there were no facilities for weighing the total produce of each plot. As the rows were 10 yd. long this gave the weight of one-tenth of the total yield. The results are given in Table II.

Table II
Yield of Exp. 1, 1935, in oz. per 10 yd. length of row

	A 179	D 253	C 201	E 277	B 234	Total 1144
	C 232	A 224	E 260	B 189	D 295	1200
	B 171	E 183	D 170	A 190	C 268	982
	D 201	B 197	A 188	C 265	E 294	1145
	E 252	C 238	B 183	D 171	A 193	1037
Total	1035	1095	1002	1092	1284	5508
	Treatments					
Treatment totals	A 974	B 974	C 1204	D 1090	E 1266	S.E.
Oz. per 10 yd. length of row	194.8	194.8	240.8	218	253.2	± 12.71
Cwt. per acre	57.4	57.4	70.9	64.2	74.6	± 3.74

The plots which were sprayed every week (E) gave 17.2 ± 5.27 cwt. per acre more than the unsprayed (A). This represents an increase of

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about 30 per cent. Plots sprayed only in June (C) gave an increase of 13.5 ± 5.27 which is just significantly above A, but not significantly different from D (sprayed only in July).

IV. EXPERIMENT 2, 1935-6

A. ARRANGEMENT AND TREATMENTS

Exp. 2 was part of a narrow strip of *Hyoscyamus* situated at the east end of the Meppershall valley and separated from the 1934 plots by a 500 yd. belt of barley and oats. The total area was only 2 acres and it was arranged in 21 long rows so that the site was not suitable for a Latin Square arrangement. The experiment was therefore arranged in

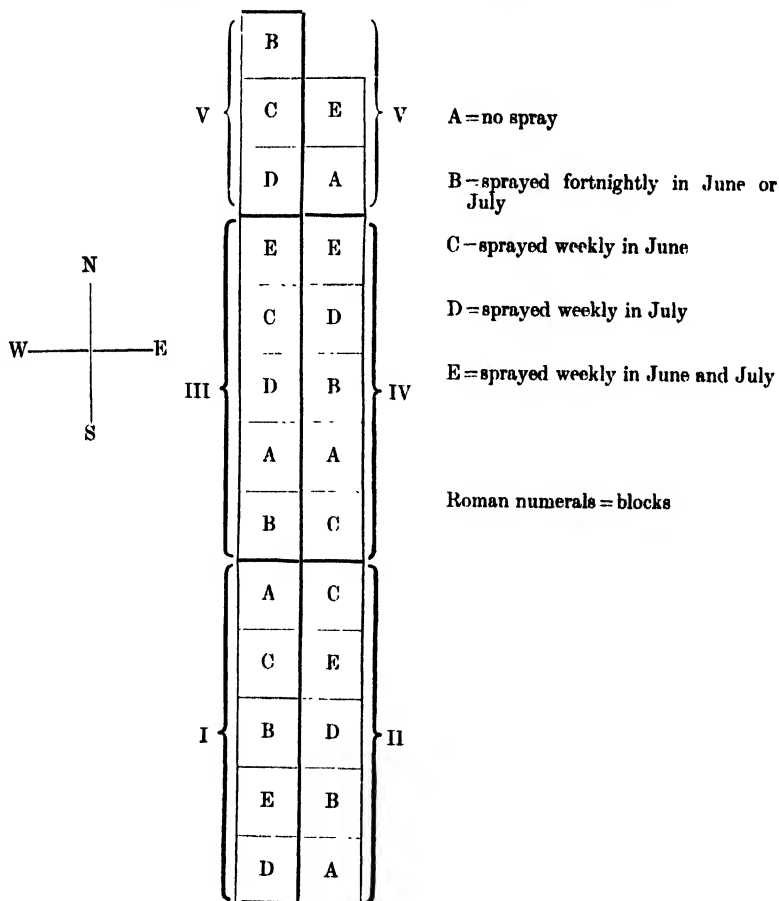


Fig. 2. Arrangement and treatments in Exp. 2.

randomized blocks each plot being 12 yd. long and 8 rows wide, so that the area covered was about the same as in Exp. 1. The remaining rows were used as guards, and spaces were also left across the rows between the plots. The slope of the ground was considerably steeper than on the 1934 site, and appeared to have an effect upon the germination rates. All the plants germinated late, the first appearing during the last week in May. No spraying was therefore possible in May, so the plots laid out for May spraying (B) were given fortnightly spraying throughout June and July instead.

The treatments and arrangement are shown in Fig. 2.

B. RESULTS

(1) *Duration of spraying*

Germination was later than in 1934 and was also irregular. Blocks I and II and the lower ends of blocks III and IV were ready for spraying on 30 May, whereas block V did not appear above ground till the following week. Spraying was continued till 19 July. The B plots were first sprayed on 7 June. The inequality in plant size due to differences in germination date affected all the first year observations and these effects were reflected in those of the following year.

(2) *Observations, 1935 (first-year crop)*

(a) *Aphis counts.*

Counts were made in the same way as in 1934 but were started as soon as aphides appeared on the plants and continued weekly for 9 weeks. Aphides appeared almost as soon as the plants were above ground, and were present on blocks I-IV before block V germinated. Counts were made on these four blocks on 31 May, when 200 plants were examined. Of these, 43 were sparsely infested with, altogether, 24 adults and 41 nymphs. These counts are omitted from Table III in which the other results are given.

Although the aphides appeared at an earlier date than in Exp. 1, and the numbers per plant were actually higher in the third week of June than in the corresponding period of the preceding year when infestation had only just started, the numbers did not increase to the same extent. It is possible that heavy rains which fell during this week made the plants unsuitable as food. The outer leaves, on which the aphides habitually feed, were found at this time to be very bedraggled and muddy. The inner leaves are sticky and hairy and aphides are rarely found on them. This caused a reduction in numbers between 21 and

28 June (see Table III) and a corresponding reduction in the peak infestation on 5 July.

Table III
Aphis counts for Exp. 2, 1935

	Date of count	Treatments (means of 5 plots)					s.e.	Mean of 25 plots
		A	B	C	D	E		
Plants infested (out of 10)	7 June	5.0	5.2	5.0	4.4	4.2	±0.781	4.95
	14 "	6.0	3.8	3.8	5.2	5.6	±0.903	4.88
	21 "	5.8	3.2	2.6	5.4	5.6	±0.816	4.65
	28 "	3.6	4.4	2.4	3.4	3.6	±1.268	3.48
	5 July	8.6	5.2	5.6	6.4	5.0	±0.22	6.16
	12 "	4.0	3.6	4.4	1.4	3.8	±1.076	3.44
	19 "	4.6	2.0	3.4	2.6	2.0	±0.542	2.92
	26 "	2.4	1.2	2.2	0.6	2.2	±0.161	1.72
	Mean	5.00	3.57	3.67	3.67	4.00	±0.282	3.98
No. of adult aphides per plot (10 plants)	7 June	5.2	3.4	4.2	3.0	2.0	±1.252	3.56
	14 "	5.2	2.0	1.0	2.6	5.0	±1.623	3.16
	21 "	14.2	5.6	1.4	6.4	6.6	±2.560	6.84
	28 "	2.4	2.6	1.6	2.8	2.0	±1.958	2.28
	5 July	9.6	4.0	3.6	7.8	4.0	±1.529	5.80
	12 "	4.8	2.8	3.0	1.8	3.0	±1.083	3.08
	19 "	3.4	1.4	3.0	0.4	1.8	±0.972	2.40
	26 "	4.0	0.8	2.2	0.4	1.0	±0.187	1.68
	Mean	6.10	2.32	2.50	3.15	3.17	±0.584	3.44
No. of nymphs per plot (10 plants)	7 June	12.6	18.8	12.4	15.0	8.1	±3.650	13.38
	14 "	18.0	5.2	4.4	10.0	18.8	±4.702	11.28
	21 "	16.4	13.6	6.0	13.6	7.8	±3.456	11.48
	28 "	6.4	9.0	3.2	11.4	8.4	±2.933	7.68
	5 July	27.6	14.4	8.1	15.1	10.8	±4.385	15.20
	12 "	11.8	6.0	11.0	3.0	5.6	±4.151	7.48
	19 "	11.0	5.8	7.4	5.2	5.6	±2.157	7.00
	26 "	3.4	3.2	4.8	2.4	6.2	±3.290	4.00
	Mean	13.57	9.50	7.16	9.54	8.91	±1.428	9.736

Italics = plots sprayed on this date.

Most of the individual weekly counts show a reduction in aphid number and plant infestation due to spraying. This is not significant for all the individual weekly counts, but the totals for all occasions show significant differences between results from sprayed weekly, and unsprayed plots. There are no significant differences between the different spraying treatments. The decrease in accuracy from that of the previous results is probably due to the smaller numbers of aphides, and less efficient experimental design. The differences between blocks are not given in Table III, but there was a significantly greater number of aphides and higher plant infestation on plots at the lower end of the field (blocks I and II) than on those at the top (block V).

(b) Estimation of virus infection.

As the inoculation method of virus estimation had not proved satisfactory, the estimates for Exp. 2 were made by observation in the field. Several independent observers took part in the survey which consisted in selecting at random 10 sections of 1 yd. length from the rows of each plot. The number of plants and the number of infected plants were recorded for each sample and some attempt was made to distinguish between "severe" and ordinary infection. This could not successfully differentiate between Hy. II and Hy. III, for in the field an Hy. II infection of long standing may cause symptoms as severe as those of more recent Hy. III, and apart from severity the symptoms are similar. The results of the survey are given in Table IV.

Table IV
*Percentage infection. Exp. 2, 1935. Estimated from
250 samples of 1 yd. length*

Treat- ments		Blocks					Total
		I	II	III	IV	V	
A	No. of plants	147	128	170	143	149	737
	Moderate infection	19	30	28	17	4	98
	"Severe" infection	11	5	2	5	7	30
	% infection	20.4	27.3	17.7	15.4	7.4	17.37*
B	No. of plants	140	127	156	158	201	782
	Moderate infection	30	25	23	10	4	92
	"Severe" infection	2	1	4	4	6	17
	% infection	22.9	20.5	17.3	8.9	5.0	13.94*
C	No. of plants	140	186	135	107	216	784
	Moderate infection	20	20	19	15	3	77
	"Severe" infection	6	4	0	3	10	23
	% infection	18.6	7.5	14.1	16.8	6.0	12.76*
D	No. of plants	148	142	158	126	147	721
	Moderate infection	21	18	11	15	10	75
	"Severe" infection	5	7	6	1	0	19
	% infection	17.6	17.6	10.8	11.1	6.8	13.03*
E	No. of plants	130	136	202	147	109	724
	Moderate infection	16	0	13	14	2	45
	"Severe" infection	0	6	6	2	0	14
	% infection	12.3	4.4	9.4	10.0	1.8	6.77*
Total	No. of plants	705	719	821	681	822	3748
	No. of infections	130	116	112	86	46	490
	Mean % infection	18.4	16.1	13.6	12.3	5.6	13.07

* S.E. = ± 1.913 .

The main treatment effect was again between the unsprayed plots (A) and those sprayed every week (E). The number of virus infected plants on the E plots was significantly below those for the other treatments.

Spraying in June and July (C and D) appeared to give a lower percentage infection than A but the difference is not quite significant. Fortnightly spraying (B) did not do much better than A. There was a definite difference between blocks; those at the lower end of the field had a higher percentage infection than those at the upper end. Thus there was agreement, for both blocks and treatments, between the estimated percentage infection and the aphid counts, which also showed an increase on the unsprayed plots and on the plots situated at the lower end of the field.

(c) *Yield.*

The yield for 1935 was obtained by weighing each plot as a whole, as the weights were within the scope of a portable balance. The results are given in Table V.

Table V
Weights in lb. per plot

Blocks	Treatments					Total
	A	B	C	D	E	
I	129	149½	110½	148	145½	682½
II	118½	108½	104½	123	86½	541
III	112½	102½	95	109	125½	544½
IV	89½	83½	86	82½	89½	431
V	65½	83½	65	71	66	351
Total	515	527½	461	533½	513	2550

No difference was found between the weights of differently treated plots, but there was a very large difference between the different blocks, presumably due to the later germination of the plants on blocks IV and V (see p. 565). However, as the heaviest aphid infestation was found on blocks I and II, which also had the highest percentage infection, there exists the apparent anomaly that aphid infestation, percentage infection, and yield are all positively correlated. This does not apply to the treatment totals, for which there is no indication of correlation.

Presumably blocks I and II had the highest percentage virus infection because these plants germinated first and became infested with aphides before those of block V. They were larger for the same reason. As the infestation was of comparatively short duration over the whole experiment, the infections were too recent at the time of cropping, to have any effect on the yield.

Yield, therefore, was only affected by position on the field, and the effect is shown in the differences between the block totals.

(3) *Observations, 1936 (second-year crop)*(a) *Aphis counts.*

No aphides were found on the plants at the time of cropping, 28 May 1936.

(b) *Estimation of virus infection.*

The percentage infection was estimated in the same way as in 1935. No distinction was made between "severe" and ordinary infection, because the symptoms were much more varied than in the previous year and it was unsatisfactory to use even an arbitrary classification. The range was so wide that it was often impossible to decide whether a plant was virus infected or merely senescent, and many possible infections were not recorded.

It seems, therefore, that neither of the methods used for virus estimation was entirely satisfactory though in all probability the infections observed in 1936 were, in fact, those responsible for the reduction in yield as the correlation between percentage infection and yield was significant. In Exp. I, percentage infection as estimated in 1934 and 1935 bore no relation to yield.

The results of the 1936 survey are given in Table VI.

Table VI
*Percentage infection, Exp. 2, 1936. Estimated from
250 samples of 1 yd. length*

Treat- ments		Blocks					Total
		I	II	III	IV	V	
A	No. of plants	89	60	125	82	88	444
	Infected plants	27	26	20	19	23	115
	% infection	30.3	43.3	16.0	23.2	23.1	25.90*
B	No. of plants	125	93	119	101	132	570
	Infected plants	29	21	15	16	32	113
	% infection	23.2	22.6	12.6	15.8	24.2	19.82*
C	No. of plants	87	81	132	86	113	499
	Infected plants	24	23	14	11	17	89
	% infection	27.6	28.4	10.6	12.8	15.0	17.84*
D	No. of plants	70	80	141	102	136	529
	Infected plants	16	16	14	22	16	84
	% infection	22.9	20.0	9.9	21.6	11.8	15.88*
E	No. of plants	112	67	148	103	80	510
	Infected plants	25	9	9	9	21	73
	% infection	22.3	13.4	6.1	8.7	26.3	14.31*
Total	No. of plants	483	381	665	474	549	2552
	Infected plants	121	95	72	77	109	474
Mean	% infection	25.1	24.91	10.82	16.24	19.85	18.57

* S.E. = ± 2.26 .

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The differences due to the 1935 treatments were still observable and so, also, were the differences for position on the field (blocks I-V).

(c) *Yield.*

The yield for 1936 was estimated from the weights of 250 random samples of 1 yd. length. The results are given in Table VII.

Table VII
Yield of Exp. 2, 1936 in oz. per 10 yd. length

Blocks	Treatments					Total
	A	B	C	D	E	
I	298	264	189	209	307	1267
II	88	132	208	173	202	803
III	278	308	316	326	364	1592
IV	268	323	272	319	357	1539
V	184	267	234	252	238	1175
Total	1116	1294	1219	1279	1468	± 82.05 S.E.
Yield in cwt. per acre	65.75	76.24	71.82	75.36	87.29	± 9.64 S.E.

The differences between individual treatments were not quite significant but E was significantly higher than A, and than the mean of A, B, C and D. Also A was significantly lower than the mean of B, C, D and E. The total yield was slightly higher than that of Exp. 1, 1935, but the percentage increase for spraying continuously every week was almost the same, i.e. 29.9 per cent in Exp. 1, and 32.4 per cent in Exp. 2.

The differences in block totals, due to the effect of position or germination rate in 1935, had now disappeared. There was some indication that they were actually reversed, for the yield from blocks I and II was now below that for blocks IV and V. There was a negative correlation for blocks and treatments between percentage infection and yield.

V. DISCUSSION

The most important result of these experiments is that an increase in final yield was obtained as a result of spraying every week during the first few months of growth. In both experiments the increase was equivalent to one-third of the yield from the unsprayed plots, or about 1 ton per acre. Other treatments such as fortnightly spraying or spraying in June or July separately, did not show significant individual increases in yield, but the results were consistently above the no-spraying level. In experiments such as these, consisting of small neighbouring plots, it is not probable that such discontinuous treatments would be very success-

ful, because the longer the intervals between treatments the greater the danger of re-infestation with aphides from surrounding unsprayed plots. If the whole of an aphis-infested area were given one of the discontinuous treatments the effect might be greater.

There was no effect on the yield at the first cropping. This was presumably because the aphides did not appear on the plants until within a few weeks of cropping, so that at this time most of the plants would be too recently infected for the yield to be seriously affected. No information is available as to the effect of spraying treatments on the yield at the second crop taken in the first year of growth.

It has been assumed that the effect of spraying was due to the reduction of virus infection by the partial elimination of the insect vectors. The numbers of aphides were not sufficiently high to have affected the plants directly, and the only well-known insect pest of *Hyoscyamus*, the *Hyoscyamus* flea-beetle, *Psylliodes hyoscyami* (4) which destroys the young plants, was not common on any of the experimental plots. Also insect damage is generally direct, and might be expected to affect the yield at the first cropping instead of the third.

Furthermore, the change from a positive correlation between percentage infection and yield in the harvest of 1935 to a negative correlation in that of 1936 (Exp. 2), suggests that the plots which had most virus infection in the first year gave the lowest yield in the second, although in the first year many of the heaviest yielding plots had a high percentage of virus infection. This is most easily explained on the assumption that reduction in the final yield was actually dependent upon disease contracted at an earlier stage of growth.

As the percentage infection estimates were not strictly comparable between the two experiments, it is better to compare them by the effects of spraying on yield. From this it appears that the loss from virus infection was approximately the same in both, although in Exp. 2 the numbers of adult aphides were well below the index figure of 100 per 100 leaves suggested by Davies (1) as necessary for rapid increase of virus infection in potatoes. It is probable that not only the maximum intensity of infestation, but also the date of arrival, and particularly the origin of the aphides are of importance in determining the severity of virus attack. Thus Hy. II virus affects many more plants than Hy. III virus, but it is less easily transmitted (p. 559). This suggested that Hy. II is readily introduced from some common source such as potatoes, which are not susceptible to Hy. III, and that the source from which Hy. III is derived, is comparatively rare.

On the other hand, the second- and first-year plants are grown in plots situated near together. The second-year plants appear first above ground. They generally have a high percentage of virus infection and are cut down just when the first-year plants are at their most susceptible stage. If, as happens in most years, they are also heavily infested with aphides at the time of cropping, there is obviously a danger of both viruses being carried on continuously from year to year.

It is suggested that the following measures could be taken to reduce the spread of virus infection, and increase the final yield:

(1) Spraying at regular intervals of not less than 1 week from the onset of aphid infestation, which can be determined by examination of random samples in the field, until the first cropping.

(2) Altering the cropping arrangements so that all the plants in one area are at the same stage of growth. This would involve sowing in alternate years instead of every year as hitherto.

(3) Keeping *Hyoscyamus* plots as far as possible from potatoes, and not using potatoes, or other susceptible plants as rotation crops.

VI. SUMMARY

1. Aphid infestation of the first-year's growth of *Hyoscyamus niger* grown as a biennial crop, was reduced by spraying with a solution of nicotine and soft soap for the first 8 or 9 weeks.

2. The greatest effect was obtained by spraying at weekly intervals, but spraying fortnightly and spraying weekly in June or weekly in July, also slightly reduced the infestation.

3. The percentage of infection by aphid-transmitted viruses was lower on the sprayed plots than on the unsprayed.

4. No effect of treatments on yield was obtained at the first cropping in the first year of growth which coincided with the end of the spraying period.

5. As a result of weekly spraying in the first year a 30 per cent increase of yield was obtained in the third crop taken in May of the second year.

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THE SOIL-BORNE FUNGUS DISEASES OF FIELD AND PLANTATION CROPS: A REVIEW OF EXISTING CONTROL METHODS

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THE problem of controlling the parasitic fungi causing root-disease in field and plantation crops is one which is common to temperate and to tropical countries alike. To emphasize the widespread occurrence and importance of such diseases, it is necessary to mention only a few examples, such as the root-rots of rubber in Malaya, of tea in India, of cotton in the Sudan, of coffee in East Africa and of cacao in West Africa, of bananas in the West Indies, and of wheat in Canada and Australia.

Fungus diseases of the aerial parts of plants can be controlled by spraying and dusting, whereby the surfaces of the plant are covered with a protective layer toxic to the germinating fungus spores. This protective layer requires renewal, its durability depending upon climatic conditions and other factors [1]. Even tree-crops 60 ft. high can be dusted by modern machines, as in the control of powdery mildew of the rubber tree by sulphur-dusting in Malaya [2]. Another striking innovation, still in the experimental stage, consists in the use of aeroplanes for sulphur dusting against cereal rusts in Canada [3].

No such simple and general method of control has been devised against the fungi causing root-diseases of plants, nor does it appear likely that this is even possible. The difficulties in the way of control are not only technical, but also economic, and any fungicidal treatment of the soil on a large scale is generally out of the question on account of the expense involved, even if there were not frequently other objections to the use of soil fungicides. Soil treatments which can be economically applied to highly paying glass-house crops are generally much too expensive for field and plantation crops; methods which are technically possible under the intensive and artificially controlled conditions of glass-house cultivation can find no place in the field.

The ideal method for the control of soil-borne diseases, as of plant-diseases generally, is by the production of resistant varieties of the crop-plant. The production and dissemination of resistant varieties is still more important in such countries as India and Egypt, where much of the agricultural production is in the hands of peasant cultivators, who generally have neither the training, the intelligence, nor the resources to employ more complicated or expensive methods of disease-control. Unfortunately, however, the selection of varieties resistant to disease is generally possible only for the more highly specialized parasites with a very limited host-range.

It is perhaps fortunate for those engaged in crop-production that the majority of the more seriously parasitic root-infecting fungi appear to be so specialized in their mode of life that they cannot multiply on the

soil humus, but can only increase on and in the host-plant. In the absence of appropriate host-plants, therefore, they may be able to persist for some time in the soil, but cannot increase; in the continued absence of host-plants, their decline and eventual disappearance is inevitable. The specialization of these root-parasitic fungi has been fully discussed elsewhere [4]; it is therefore proposed to examine here only its practical implications.

With this in mind, a classification of existing control methods may be made as follows:

1. Methods which are designed to get rid of the fungus during its passive phase in the soil in the absence of host-plants.
2. Methods which check the subterranean activity of the fungus during its active parasitic phase on the roots or other underground parts of the growing crop.
 - (a) Measures designed to increase resistance to attack of the underground organs of the host, where this is possible.
 - (b) 'Rogueing', i.e. the removal of infected plants or parts of plants from the ground in an endeavour to check the subterranean spread of the fungus parasite from infected roots, &c., to healthy roots in contact with them.
 - (c) Measures intended to make the soil environment less favourable for the parasitic activity of the fungus.
3. Methods intended to eliminate as far as possible the dispersal of the fungus to fresh areas by outside agencies, e.g. wind, water, insects, animals, and agricultural practices.

It is not proposed to discuss here in further detail the methods included under the third category, since dispersal may be effected by very varied agencies, and methods of control will obviously vary widely according to the peculiar circumstances of each individual case. Further discussion will therefore be reserved for methods included under the first two categories.

Methods Designed to Eradicate the Fungus during its Resting Phase in the Soil

It will be readily apparent that the problem of root-disease control is more difficult to solve in a permanent or so-called plantation crop, where the parasitic phase of the fungus can last unbroken, than in an area under annual cropping. In the latter case, the period between successive susceptible crops, during which the parasite must enter upon a passive or resting phase, represents a weak point in the economy of the fungus.

The fungus parasite may persist from one susceptible crop to the next in the form of resting spores or other especially adapted survival organs, such as sclerotia, or in infected roots and stubble residues left in and on the soil after the diseased crop. The established method for eliminating such infection from the soil is that of crop-rotation, whereby no susceptible crop is grown for a period sufficient to allow most of the infection to die out from the soil. Reduction of infection to a very low

level, rather than complete eradication of the parasite, generally constitutes a satisfactory compromise.

Since infection has not infrequently been found to survive in the soil for periods of as much as five years after a heavily diseased crop, control by crop-rotation is not always very satisfactory. It is possible, of course, that such survival may sometimes be only apparent, being really attributable to the carriage of the fungus parasite on susceptible weed-hosts during periods of fallow and alternate crops, or to reinfection of the land from outside sources. Nevertheless, a general search has been made for methods whereby the death of the fungus in such infected soil may be artificially accelerated. Under the intensive system of glass-house cultivation, steam-sterilization of the soil is very generally practised [5]; one serious disadvantage of this method is that the soil is rendered much more favourable for the renewed activity of the fungus parasite, if accidentally reintroduced through infected plants or cuttings, subsoil, walls of beds, or pots. Both for field and glass-house crops, chemical sterilization of the soil has been not infrequently advocated. Experimental field trials have rarely given satisfactory results, though the method has found a certain popularity for the control of insect pests difficult to eradicate in any other way [6]. Disadvantages of soil fungicides are the expense of treating large areas, the fact that the initial concentration of the toxic chemical is rapidly reduced by the soil, and the fact that the killing agent can rarely reach fungus mycelium buried at all deeply in infected plant-residues. Yet other objections can be brought forward, but those already raised constitute a strong deterrent to the use of this method.

More attention might well be paid, therefore, to the operation of natural agencies in effecting the disappearance of parasitic fungi from the soil in the absence of their host plants. It was originally considered that a parasite disappeared from the soil solely through starvation in the absence of an appropriate host; it is now known that such disappearance may be greatly hastened by the antagonism to the parasite of the saprophytic fungi and bacteria of the soil, which attack and decompose it during its resting phase. It has been shown experimentally that this antagonistic action of the soil saprophytes can be increased in various ways; their activity is especially accelerated by the addition of suitable organic matter to the soil, on which they can feed and multiply. It seems very possible that, with further experimental work, such expedients can be adopted as a means for shortening the period of fallow or alternate cropping necessary for the eradication of a fungus parasite from the soil after a diseased crop. Such a method can be fitly termed 'biological control'.

In certain classes of root-infecting fungi, e.g. the smuts and *Fusarium* seedling-blight of cereals, survival in the soil from one season to the next may often be inappreciable, but the fungus is carried over the break from one crop to the next on or in the seed; under local conditions unfavourable to the survival of soil infection, therefore, control of such seed-borne diseases can be obtained by seed-treatment with a disinfectant dust or steep, or with hot water. The practice of seed-treatment,

with especial reference to cereals, has been recently reviewed by Leukel [7].

In permanent or plantation crops, on the other hand, there is no such break in the continuity of the parasitic existence for a root-infecting fungus. Greater attention must therefore be paid to securing a clean start on land destined for a plantation crop. Especially is this the case with tropical crops, which are more often than not planted on the site of cleared jungle. Root-disease trouble in developing plantations is generally a direct legacy from the original jungle. The root-disease fungi exist in biological equilibrium with their hosts in the natural jungle, and nowhere is infection very apparent. This equilibrium is destroyed by the process of clearing and felling the jungle, and if a uniform population of susceptible hosts, in the shape of the plantation crop, then be planted on the cleared site, the result may be a spread of one or more root-disease fungi under peculiarly favourable conditions. Such troubles would not arise, of course, were the land laid down to non-susceptible annual crops for a period after clearing. This is apparently rarely practicable, however, and in the past the method adopted has generally been that of laboriously extracting as many as possible of the diseased roots and stumps of the jungle timber before planting the crop. Much of our knowledge of this sequence of events derives from recent work carried out at the Rubber Research Institute of Malaya [2]. In Malaya, the laborious process of clean clearing for the control of rubber root-rots is being replaced by a system in which infection is gradually eradicated in the first years of the growing crop. A tree-to-tree inspection of the collar and proximal parts of the roots is made at four-monthly intervals; where infection is discovered, it is traced back to the piece of infected jungle timber which was its original source; this is forthwith removed and destroyed, and the infected root appropriately treated or destroyed, according to its condition. In this elegant method of control, the plants themselves are used to indicate the site of infected timber lying in the soil, and much expensive labour in grubbing the whole of the planting site is thereby saved. Such a control method, of course, really comes under the heading of roguing (*v.i.*).

Of very recent interest is a development in Nyasaland, due to Leach [8]. After the felling of the jungle in preparation for clearing a site for tea-planting, there is vigorous and widespread infection of the roots of certain species by the fungus *Armillaria mellea*. This fungus occurs only to a moderate extent on the roots of the living jungle, but its development seems to be greatly facilitated by the lowered resistance of the roots after felling. *A. mellea* is then later on responsible for much trouble in the young tea-plantations, which may suffer severely from its attack. The only control method previously known has been removal of the infected roots and stumps from the soil. Leach has now made the significant observation that if the jungle trees are ring-barked some time before felling, the roots are invaded not by *A. mellea*, but by certain other fungi, which are harmless in young tea-plantations. He has attributed the failure of *A. mellea* to infect the roots of ring-barked trees to its preference for roots with a normally high carbohydrate-content,

and considers that it may be unable to invade and parasitize those in which the carbohydrate-content has been seriously depleted by ring-barking. Such a restriction might be due, of course, to the competition of other organisms better able to infect such roots low in carbohydrate-content, rather than to absolute inability of *A. mellea* to colonize such a substrate.

Methods that Check Activity of the Fungus during the Parasitic Phase

(a) *Increasing host-resistance*.—In many diseases caused by root-infecting fungi, the plants affected may be growing under optimum soil conditions and in full vegetative vigour. In other cases, however, the plant may be predisposed to disease by some injurious factor in the soil environment, or its resistance may be lowered by some nutritional deficiency in the soil. When this is so, rectification of the environmental factor concerned will be found to control the disease. The serious root-rot of sugar-cane in Hawaii, the West Indies, and elsewhere [9], and the browning root-rot of cereals in Canada [10], both appear to be due in the first instance to a low phosphorus-nitrogen ratio in the soil, though the immediate cause of the trouble in each case is the fungus *Pythium arrhenomanes*. Both diseases can be controlled in the field by the application of phosphate. Again, a serious root-infection of cotton in the Sudan, which has caused much concern of recent years [11], is associated with a number of different fungi, but the predisposing cause seems to be the poor root-aeration in the very heavy soils on which the crop is grown. In conclusion, the need for further research work on the relation of host-resistance to the nutrition of the plant must be emphasized. Such knowledge as we have concerning manurial factors and the incidence of disease is concerned mainly with foliage diseases, such as potato blight, apple scab, and the rusts and mildews of cereals. In the case of root diseases, manurial treatments must operate directly upon the causal fungus as well as upon host-resistance, and the precise effect of such treatments upon the latter can therefore be evaluated only with difficulty.

(b) *Rogueing*.—The majority of the more specialized root-infecting fungi appear to spread underground not directly through the soil, but along the inside or outside of the underground organs of the host [4]. Spread from an affected plant to its neighbours, therefore, occurs in such cases only by root-contact; the removal of visibly affected plants, together with the greater part of their root-systems, might thus be expected to check the centrifugal progress of infection from scattered infection foci in the crop. This is not so simple a matter as might appear, however, owing to the great extent of the root-system, and the difficulty of recovering diseased portions of roots from the ground. Moreover, infection may have travelled underground along the root-network for a considerable distance beyond a visibly affected plant, and the removal of this alone will thus fail to check the outward progress of the fungus.

This is well illustrated by the fate of heroic efforts to check the rapid extension of Panama disease of bananas in Jamaica, which is described by Cousins and Sutherland [12] and by Wardlaw [13]. The progress

of the disease, first reported on the island in the year 1911, was considerably checked, though not definitely arrested, by early enforcement of drastic rogueing, whereby it was compulsory to uproot and destroy plants growing on an area of four square chains around every visibly wilted plant. With the more rapid extension of the disease after the year 1920, the four-square-chain-system of rogueing was replaced by a graduated system, as follows: first to third case, four square chains; fourth to fifth case, one square chain; and after the fifth case, half a square chain. Finally, in some parishes, the disease became so widespread as to necessitate the reduction of the quarantine area to the individual visibly diseased plant, and the eight apparently healthy ones surrounding it. This is referred to as the nine-root treatment. The failure of rogueing to do more than merely retard the extension of Panama disease in Jamaica must be attributed in large part to the spread of the causal organism, *Fusarium cubense*, by agencies other than the host-plant. For example, the transport of infected planting material to disease-free areas must have been frequently responsible for the appearance of the disease in fresh localities, whilst Wardlaw [13] considers that flood-waters may have played an important part in the natural dissemination of the parasite.

More satisfactory appears to be the state of affairs on Malayan rubber plantations, where the rogueing method is being established on a firm basis by a thorough investigation of the whole root-rot problem [2]. By means of the periodical tree-to-tree inspection (described above), guess-work is being eliminated, and a gradual eradication of all buried sources of infection assured.

Rogueing methods have so far found their chief application among the root diseases of plantation crops, where, indeed, there is often no other choice. With annual field crops, other methods, such as crop-rotation, are available and more economical, and rogueing has so far found little favour. An interesting study of the *Verticillium albo-atrum* wilt of potatoes has been published by McKay [14], who found that a considerable reduction in the percentage of infected tubers at harvest could be achieved by the 'three-plant method' of rogueing, whereby a visibly wilted plant was pulled together with its two immediate neighbours in the row. Infection by root-contact appeared to spread much more rapidly along than across rows; this was attributed by McKay, not so much to the greater distance apart of plants between than along rows, as to the fact that root-development and root-contact between the rows had been checked by frequent cultivations during the earlier part of the growing season.

(c) *Making soil conditions less favourable for the subterranean activity of the fungus.*—Certain root-diseases of crops show a well-marked distribution with soil type; some are found on acid soils, but not on alkaline soils, and vice versa; others are limited to soils of light texture, and others again found only on soils of heavy texture, and so on. It is thus possible to some extent to tabulate these different root-diseases according to soil type [4]. Amendment of soil conditions, in such a way as to hinder the activity of the parasite, thus suggests itself as an aid in the control of

disease. Whilst this is certainly more easily accomplished in glass-house cultivation [15], there is yet appreciable scope for application of this principle under ordinary field conditions.

Even with field crops, some control of soil temperature can sometimes be obtained by variation in the time of planting [16]; control of wheat bunt, for example, is assisted by early autumn or late spring sowing, since at soil temperatures of 20° C. and above the wheat seedlings germinate more quickly and may escape infection [17]. Again, Walker and Wellman [18] found that onion smut, due to *Urocystis cepulae*, did not occur in those parts of the United States in which the soil temperature at planting time exceeded 29° C.

Soil tilth and aeration can certainly be varied by tillage operations. Thus Griffiths [19] has recommended the compaction of light soils by implements and by the trampling of a flock of sheep as a valuable control measure for the take-all disease of wheat.

Effective control of certain diseases, viz. potato scab [20], cotton root-rot [21], and take-all of wheat [22], has been obtained by the application of organic manures. Such control has been attributed chiefly to the antagonistic action towards the parasite of the soil saprophytes, the activity of which is greatly increased by the addition of fermentable organic matter to the soil. Such antagonistic action, in all probability, not only hinders the parasitic activity of a root-infecting fungus during the growing-period of the crop, but also hastens its disappearance from the soil during the subsequent fallow period.

Certain of the root-infecting organisms are very sensitive to soil reaction. The control of potato scab by the application of inoculated sulphur, to bring about an acid soil reaction, and the control of club-root of crucifers by liming, are widely quoted examples of the effect of soil reaction. Whilst quite a number of soil-borne diseases have been found to be potentially controllable by adjustment of the soil reaction, such a measure is only practicable on the more lightly buffered soils.

Control of disease by soil amendment is only possible within the range of soil conditions permitting economic production of the crop. Many of the root-infecting fungi show a tolerance of different soil conditions almost as wide as that of their host-plants; others, again, are most active under just those soil conditions optimum for crop production. Even where control of a disease by soil amendment is possible, however, it will frequently prove impracticable; it can rarely be economically feasible, for instance, to change appreciably the reaction of highly buffered soils.

Where soil amendment is not possible, it may still be feasible in some cases to restrict the production of the crop to soil types unfavourable to disease. This possibility applies more particularly to the case of permanent or plantation crops, where for obvious reasons soil conditions are less under the control of the grower than on land devoted to annual crops. Of especial interest in this connexion is the work of Reinking [23], who has demonstrated a clear relationship between the incidence of Panama disease of bananas and soil type; the disease was found to be most prevalent on the light-textured sandy soils. Such considerations

should be borne in mind by those taking up land for the cultivation of a tropical plantation crop. Indeed, it may well be in the field of prevention rather than in that of cure that the study of soil conditions and soil-borne fungus diseases will eventually find its most valuable application.

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SOIL FUNGI AND ACTINOMYCETES IN RELATION TO MANURIAL TREATMENT, SEASON AND CROP

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(With Plates VI and VII, 2 Text-figures and 4 Graphs)

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I. INTRODUCTION

IN recent years it has become increasingly recognized that the fungi and actinomycetes constitute an integral part of the soil microflora and play an important role in the soil economy. Their importance in various microbial processes rivals that of the bacteria and, in the aerobic decomposition of cellulosic materials in the soil, they may be the primary agents. In the study of soil mycology, however, information on such fundamental points as the effect of cropping, manuring, seasonal changes, moisture, etc., on the fungal content, is scattered, inconclusive and not infrequently contradictory. The present investigation was started with the following questions in view:

Quantitative. Whether fungi and actinomycetes in cultivated soils are affected by different manurial treatments and, further, whether there exists any correlation between their numbers and soil fertility as measured by crop growth. Whether soil fungi and actinomycetes exhibit phenomena

of periodicity as described for other soil organisms such as bacteria and protozoa. Whether different crops exert a specific influence upon the numbers of fungi and actinomycetes.

Qualitative. Whether differently manured plots harbour characteristic types of soil fungi as a result of specific treatments. Whether different crops influence the presence or absence of different types of soil fungi.

The investigations were conducted on two fields: (a) Broadbalk (continuous wheat and differential manures since 1843), (b) Barnfield (continuous roots and differential manures since 1856, mangolds since 1876).

Rothamsted soil is heavy loam with clay and flints, contains sufficient lime, and the pH value ranges from 7 to 8.

As the same crops and cultural operations are repeated each year it may be considered that the microbial population of the plots has become fairly stabilized, and that any differences which may occur in the populations of the several plots are relatively permanent and correlated with the differential treatments and crops. Certain plots in Broadbalk receive the same manurial compounds as particular plots in Barnfield, and these plots were chosen for examination. Further, within each field, these selected plots can be arranged in order of fertility as measured by the crop. The selected plots with their manurial treatment and crop yield are given in Tables I and II, where the principal operations conducted in the two fields are also stated.

In Barnfield an additional plot, 1A (dung 14 tons, sulphate of ammonia 412 lb., 21.70 tons of roots per acre), was also selected in order

Table I

Broadbalk. Wheat since 1843. Plots selected for present investigation

Plot no.	Yield in bushels per acre, av. 1852-1925	Manurial treatments
3	9.8	Unmanured
10	17.8	412 lb. sulphate of ammonia per acre
11	21.4	412 lb. sulphate of ammonia, 3½ cwt. superphosphate per acre
13	30.6	412 lb. sulphate of ammonia, 3½ cwt. superphosphate, 200 lb. sulphate of potash per acre
2 B	34.2	Dung 14 tons per acre

Red Standard wheat sown 16 October 1930. Manured 27-28 March 1931. Cut 17 August 1931. Field ploughed with tractor 29 August to 15 September 1931.

Table II

Barnfield. Mangolds since 1876. Plots selected for present investigation

Plot no.	Yield in tons of roots per acre, av. 1876-1928	Manurial treatments
8 O	3.34	Unmanured
8 A	5.32	412 lb. sulphate of ammonia per acre
5 A	6.70	412 lb. sulphate of ammonia, 3½ cwt. superphosphate per acre
6 A	13.50	412 lb. sulphate of ammonia, 3½ cwt. superphosphate, 500 lb. sulphate of potash per acre
1 O	17.47	Dung 14 tons per acre
1 A	21.70	Dung 14 tons, 412 lb. sulphate of ammonia per acre

Mangold variety, Yellow Globe; swede variety, Purple Top. Dung applied 14-15 November 1930. Tractor cultivation 26-27 March 1931. Manures applied 10 April 1931. Mangolds sown 15-17 April 1931. Crop failed. Field rolled 21 May 1931. Swedes sown 26 May 1931. Nitrogenous top dressing 17-18 July 1931. Roots lifted September 1931.

that the effect of heavy nitrogen dressing might be examined. Plot Barnfield 6 A thus received 2.5 times as much sulphate of potash as plot Broadbalk 13, but afforded the closest comparison that could be made in the two fields.

II. SURVEY OF LITERATURE

Quantitative. It is only comparatively recently that soil fungi and actinomycetes have been considered as other than minor soil organisms, with the result that their study has been subsidiary to that of bacteria. Perhaps the chief impediment was the lack of any standardized technique before that evolved by Brierley, Jewson & Brierley (1). Earlier workers, Remy (23), Ramann (22), Goddard (13), Waksman (26-34), Taylor (25) and Jones & Murdock (17) concerned themselves with the estimation of relative numbers of fungi and actinomycetes in a wide variety of soils, and their work shed little light on the specific effect of soil treatments and other factors upon the numbers present.

Brown & Halverson (4) in the U.S.A. made an extensive study, extending over a whole year, of the effect of seasonal conditions and soil treatment on bacteria and moulds, their results showing that "the numbers of moulds present in the soil fluctuated from one sampling to the next but were apparently unaffected by moisture, temperature or soil treatment. Some factor uninvestigated probably accounts for the change".

Erdman (9), in his studies of the numbers of fungi and actinomycetes in Cardington loam as influenced by different treatments, found that

"in the great majority of cases the soils from the manure and lime-treated plots showed practically the same number of fungi as the soil from the check plot. In fact the data showed an apparent tendency for the numbers of fungi to be smaller in these two treated soils than in the untreated soil". With regard to the actinomycetes, also, he stated that "the different soil treatments had very little effect on the actinomycetes of the soil. About the same number of these organisms was found in the soil from the manure and the manure and lime treated plots as was present in the untreated plots". Further, in no instance did his curves showing the number of fungi exhibit any relation to the crop yield.

Jensen(15), in his studies on actinomycetes in Danish soils, stated that the reaction of the soil was a more dominant factor than the soil type in determining the number of actinomycetes in soils. Also, in his description of 100 Danish soils, Jensen(16) was unable to trace a direct relation between the numbers of fungi in the soils and their agricultural fertility. He mentions, however, that a certain influence of fertilization is discernible in differently treated plots of the same soil. Shunk(24), in the U.S.A., could not find any appreciable difference in the fungal contents of differently treated soils in general and those treated with enough lime in particular. Williams(36), in England, failed to find a marked influence of stable manure upon the numbers of fungi and actinomycetes in glasshouse tomato soils.

Against such negative evidence on the response of fungi and actinomycetes to soil treatment may be set the work of other investigators who have obtained different results. Marchal(19) recorded the presence of relatively few fungi in soils that had been extensively cultivated or those soils containing a small proportion of organic matter. McLean & Wilson(20) gave figures, from studies in New Jersey, which show a decided influence of the quality of organic matter on the activity of the soil fungi. Löhnis(18), Gerlach & Vogel(12), and Butkewitsch(5), in Germany, showed the favourable influence of ammonium salts and of nitrates upon the soil fungi. Fischer(11), in Germany, found that farmyard manure increased the number of fungi in his three types of soils by two, three and five times respectively. Faelli(10) in Germany, Oudemans & Koning(21) in Holland, Hall *et al.*(14) in England and Waksman(31, 33, 34) in the U.S.A. recorded greater numbers of fungi in rich acid soils.

Studies by Coleman(7) in the U.S.A. of sterile soils showed that such factors as temperature, aeration and food supply affect considerably the activities of soil fungi. Waksman & Starkey(35) stated that the micro-organisms reach a much higher number in fertile than in less fertile

soils. Dixon (8) recorded a greater number of fungi in clay soil than in sandy soil. Further, he observed minimum counts during the cold and dry periods of the year, and the maximum after rain with sufficiently high temperature. Brierley (2), in his investigations at Rothamsted on Broadbalk plots, observed a numerical difference in fungal content in the variously manured plots, and, further, remarked (3) that "different soils vary considerably in their numerical fungal content, the more fertile soils containing the greater number".

This brief outline of the major investigations of this subject shows that the evidence is contradictory in nature and that no clear issues are presented.

III. EXPERIMENTAL WORK

(a) *Technique*

The plots, as already mentioned, were selected from Broadbalk and Barnfield and regular periodic work was started early in December 1931. The methods followed throughout were those suggested by Brierley *et al.* (1). Soil samples from the top 6 in. of the different plots were extracted laterally from a newly opened vertical soil face, and sampling was conducted throughout the year in the two fields on alternate fortnights, the samples being taken generally in the morning. 25 g. from each plot were weighed and dried for 24 hours in Petri dishes for the determination of moisture content. The hydrogen-ion concentration of the soil was determined on the same day. Slight variations were observed in the pH values but, generally, in the plots under consideration the pH value of the soil is slightly on the alkaline side, ranging between 7 and 8.

Conn's medium¹ was used, and a dilution of 1/20,000 was employed throughout.

Since it had been decided to estimate the number of actinomycetes as well as the fungi it was necessary that the pH of the medium should be such as to give the maximum number of colonies of both fungi and actinomycetes. Various hydrogen-ion concentrations from 4 to 7.2 were tested, and the value which gave the most satisfactory results was 5.2.

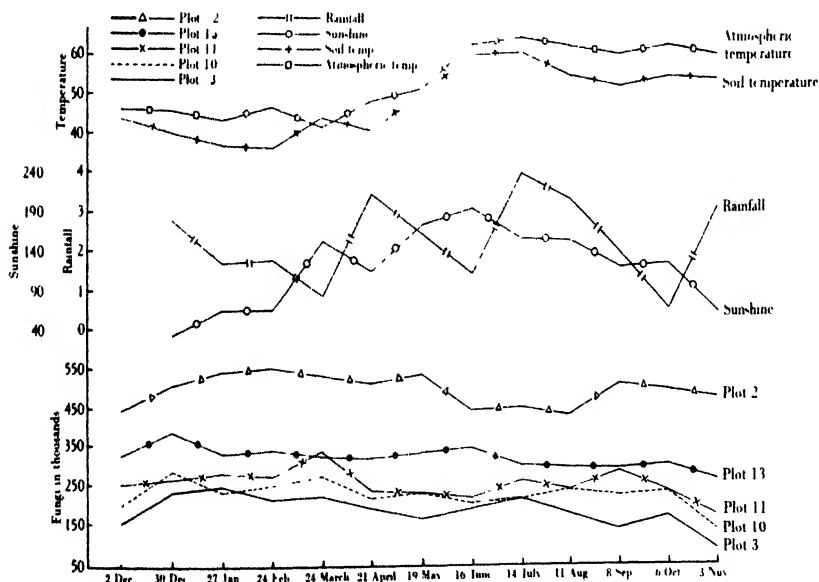
A series of eight plates in parallel was used for each plot, and 1 c.c. of 1/20,000 dilution of the soil sample used as inoculum. The plates were incubated at a temperature of 24–26° C. for a period of 7 days. In order to count the colonies on the plates the latter were examined under a strong opal electric bulb, and then a final scrutiny made with the plate

¹ Sodium asparaginate 1 g., dipotassium phosphate 1 g., glycerin 10 c.c., water 1000 c.c., washed agar 25 g.

held obliquely in various directions against the light. The counts were made on the 5th and 7th days of incubation, and the method employed was to mark on the bottom of the plate all colonies of fungi and actinomycetes with different inks (red for actinomycetes, black for fungi) on the 5th day and record the numbers. On the 7th day the plates were re-examined and all additional colonies of both types of organisms were counted and added to the 5th day figures. The two countings proved a useful check on each other. One or two plates in each series were sometimes spoiled by over-spreading forms (principally *Trichoderma* sp.), and such plates were not included in the final estimations. Preliminary work carried out to gain experience of methods made it clear that the chief essential in the technique is standardization of every minute detail and, keeping this in view, the plating method gives results which can be replicated with confidence.

(b) Results

The final estimations were made on a series of soil samples taken fortnightly at fairly regular intervals from December 1931 to November 1932, and the results are shown in Graphs 1-4 and Text-figs. 1 and 2. In Graphs 1 and 2 are also shown the rainfall, sunshine and atmospheric and soil temperatures on the two fields during this period.



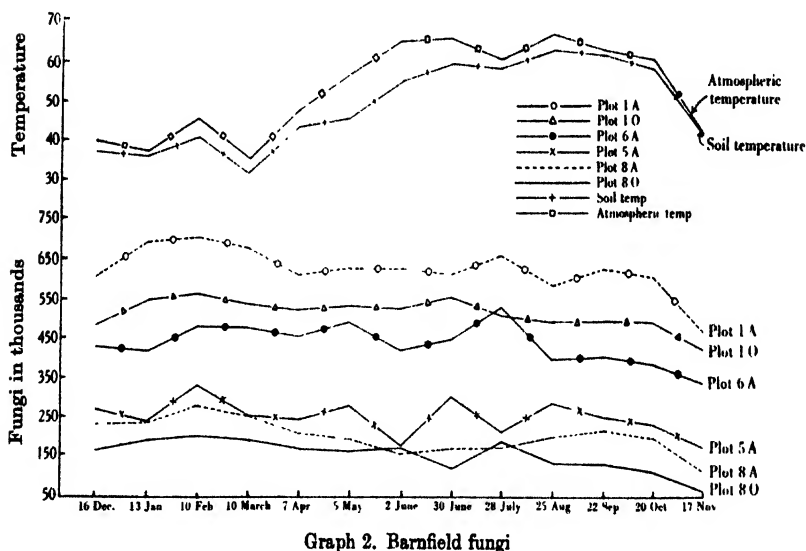
Graph 1. Broadbalk fungi

IV. DISCUSSION

The aspects of the problem may be discussed separately.

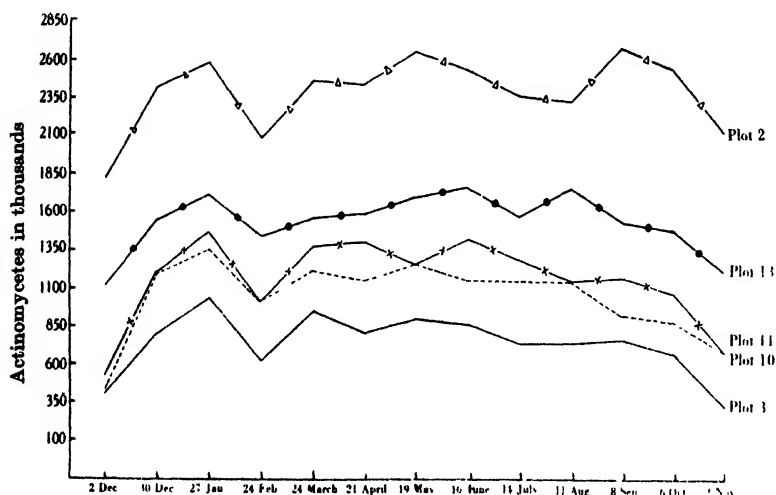
(1) *Correlation between soil fertility, crop growth and the number of fungi and actinomycetes in the soil.*

Graphs 1-4, Text-figs. 1 and 2 and Plates VI and VII show that the numbers of micro-organisms present in the soil are directly correlated with the different manurial treatments; the more fertile the plot the greater are the numbers of fungi and actinomycetes. The results, therefore, of Brown & Halverson (4), Erdman (9) and others who were not able to trace any direct effects of fertilization upon the numbers of fungi and

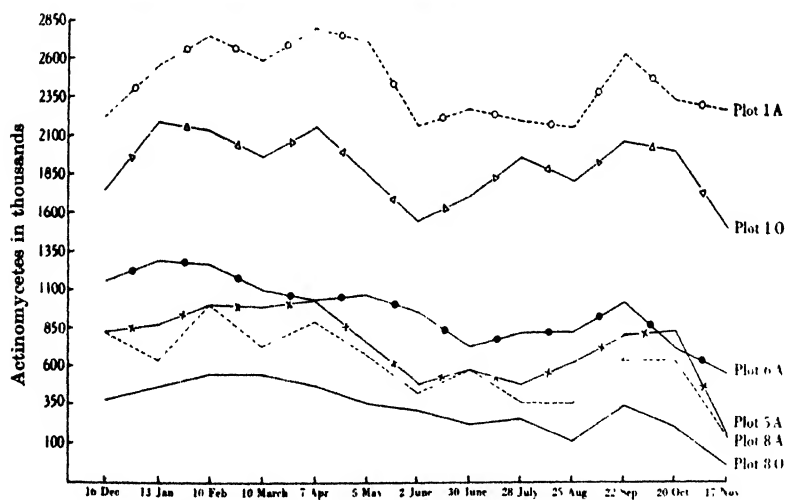


actinomycetes are not supported by the present work. It should be remembered, however, that the plots on Broadbalk-Barnfield have been subjected to differential manurial treatments for 88 and 75 years respectively. On the other hand, support is here indicated of the more general statements of Waksman (30,31), Marchal (19), McLean & Wilson (20), and Brierley (2,3). While the correlation is here obvious certain additional observations may be made. Fungi as a whole are relatively greater in quantity in Barnfield than in Broadbalk, but, on the other hand, Broadbalk harbours a relatively greater number of actinomycetes than Barnfield. The results suggest that there may be a certain potential micro-organism content and that what is lost in actinomycetes in

Barnfield is counterbalanced by the fungi, and conversely in Broadbalk. The corresponding plots, 2 B Broadbalk and 1 O Barnfield, are signifi-



Graph 3. Broadbalk actinomycetes. (Plot signs as in Graph 1.)



Graph 4. Barnfield actinomycetes. (Plot signs as in Graph 2.)

cantly greater than 13 Broadbalk and 6 A Barnfield respectively in the number of actinomycetes and fungi, but plots 1 O and 6 A Barnfield do not differ significantly as regards the fungal numbers. Plots 6 A and 13 are significantly greater than 5 A and 11 as regards the fungi but in

Barnfield the difference in the number of actinomycetes is barely significant. Plots 5 A and 11 as compared with 8 A and 10 are rather interesting. The differences in both fungi and actinomycetes in these plots, while apparent, are not of any magnitude. The crop yield, however, in these two plots does not differ appreciably, hence this fact suggests that where the fertility (crop yield) does not differ markedly the numbers are comparatively alike. Plots 8 A and 10 differ significantly from the controls 8 O and 3 in the number of fungi and actinomycetes.

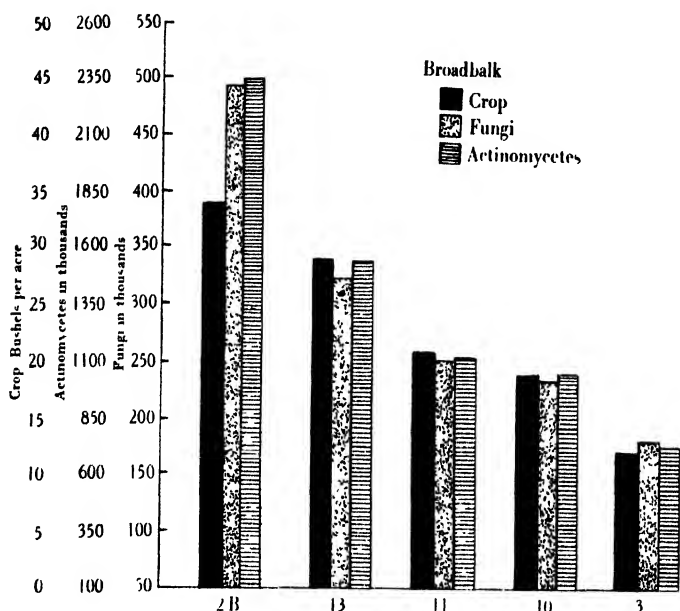


Fig. 1

In the additional plots 1 A and 1 O in Barnfield the differences both for fungi and actinomycetes only verge towards significance.

The question arises whether it is the fungi and actinomycetes which, by their activities in the soil, e.g. cellulose decomposition, ammonification, etc., make the soil more fertile or whether, because of the greater amount of nutrient added in manurial form to the more fertile soils, their numbers have increased. The answer to this problem does not ensue from the present work but the fact seems clear that the relative total numbers of fungi and actinomycetes can serve as a possible index of soil fertility.

(2) *Periodicity in the fungi and actinomycetes of the soil.*

It is clear from the work of Cutler *et al.* (6) that bacteria and Protozoa show seasonal changes, a definite periodicity with a maximum in spring and autumn and a reduction of numbers in summer and winter. Seasonal periodicities have also been recorded for both freshwater and marine algae. In considering, first, the fungi as a group, their behaviour throughout the year has been followed in both Broadbalk and Barnfield

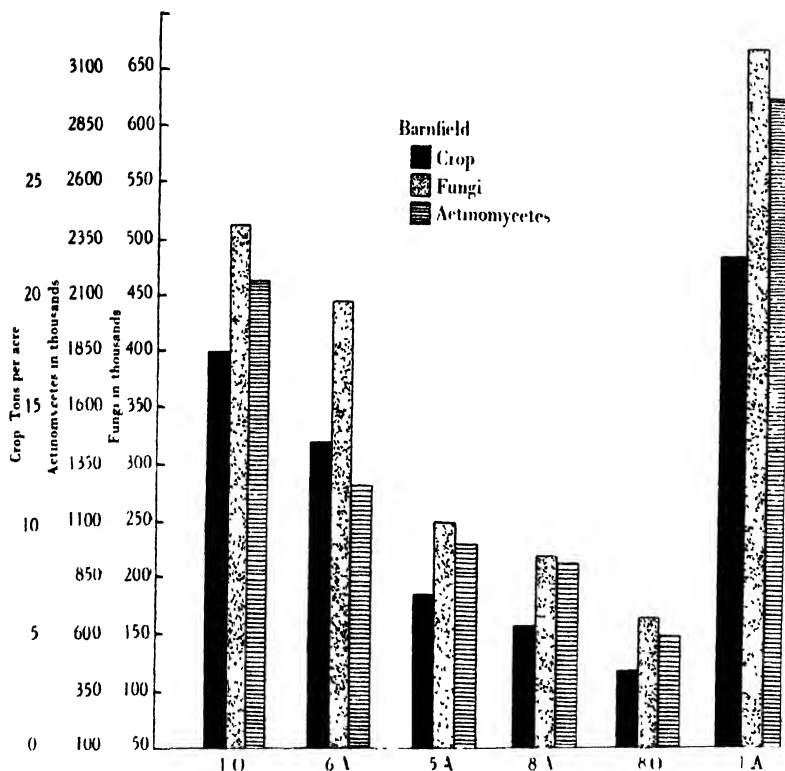


Fig. 2

and is shown in Graphs 1 and 2 respectively. It is interesting to note that, in both fields, the number of fungi throughout the year seems to have approximated about a mean value for each plot and that, during a period of 12 months, there is no apparent relation with seasonal or climatic changes.

In Broadbalk plot 2 B there is a slight depression of numbers during the summer and winter, and this plot alone might possibly suggest something of the nature of a periodic cycle, winter and summer fall with

a rise in spring and autumn, were it not that the Barnfield results completely negate it. Although the two fields are subject to different operations such as sowing, manuring, cultivation, rolling, harvesting, etc., during the whole year, it might be argued that, if seasonal changes had any dominant effect, it should have been manifested in all the plots on both the fields. Failing to get such a result one is led to the tentative conclusion that periodicity in soil fungi is not a definite phenomenon or that the methods employed are not suitable for its exhibition.

The behaviour of actinomycetes in both the fields is plotted in Graphs 3 and 4. It will be seen that marked evidence of periodicity is lacking although there would seem to be an indication of a fall in numbers during the winter months.

In general, the evidence with regard to periodicity in fungi and actinomycetes in the present study is negative or, at best, inconclusive. Unfortunately, the weather conditions during 1931, and especially during the summer months, were extremely unsatisfactory and the absence of a real and marked rhythm in weather conditions during this period of observation may be reflected in the inconclusive nature of the data obtained. Although in the results there is an indication of a fall in winter numbers, if all the data are considered together the evidence is more suggestive of a scattering of numbers about a mean value during the rest of the year. Data for other physical factors such as atmospheric temperature, soil temperature, rainfall, and sunshine are inserted in the graphs, but the fluctuations in numbers do not seem to correspond with any of the external conditions. It may be suggested that the problem of periodicity can only be finally solved if periodic samplings are taken on a number of differently manured fallow or grass plots. An experiment of this kind, which would exclude all factors such as different crops, cultivated or uncultivated fields and various field operations, would alone give conditions really suitable for a conclusive study of the phenomenon of periodicity in the soil fungi and actinomycetes.

(3) *The relation of different crops to the fungi and actinomycetes of the soil.*

Figs. 1 and 2 show the possible effect of the different crops on the number of fungi and actinomycetes. While it may be suggested that the different crops, as such, do not seem to affect the numbers in a prominent way, nevertheless the numbers of fungi are, on the whole, greater in the Barnfield than in the Broadbalk plots. On the other hand, actinomycetes are reversely affected, Broadbalk plots having greater numbers than those in Barnfield. The only important factors differing in the two

fields are the crop and the cultural operations. It would seem therefore that the balance of the two groups of organisms may be conditioned by the different plant residues in the soil or by the direct influence of the different crops, or perhaps by a combination of these factors.

V. GENERAL OBSERVATIONS ON THE QUALITATIVE DATA

Side by side with periodic quantitative estimations an examination was made of the qualitative aspects of the fungus flora of the various plots as affected by different manurial treatments and crops in both Broadbalk and Barnfield. It was impossible to identify all the numerous fungi which appeared on the plates during the course of the work, but certain important soil forms were amenable to rapid identification on account of their obvious methods of sporulation and characteristic colony formation. The following were amongst the genera selected for observation: *Penicillium*, *Aspergillus*, *Mucor*, *Fusarium*, *Dematium* forms and *Monilia*.

The data accumulated in this connexion indicate that it is not possible to state that any of the differently manured plots harbour definite or characteristic types of fungi. All the more important forms are represented in each of the several plots but the more fertile plots seem to contain, besides the dominant genera, a greater variety of forms such as species of *Verticillium*, *Trichoderma*, *Phoma*, *Cephalosporium*, *Sporotrichum*, *Sepedonium*, *Cladosporium*, *Helminthosporium*, *Macrosporium*, *Alternaria*, and sterile hyphae of various other fungi. The above negative association of particular fungi with specific manurial treatments was, however, only one aspect of this problem. The different crops or, perhaps more accurately, the residues left by different crops may have a marked effect as regards the density of the various forms, since certain types of fungi seem to be more favoured in their growth in one field than in the other. It was observed that Barnfield harbours greater numbers of the genus *Penicillium* and forms of *Dematium* while Broadbalk contains greater numbers of the genus *Fusarium*. Species of *Monilia* are common in both the fields, and *Aspergillus* spp. and *Mucor* spp. are extremely deficient in the two fields under consideration.

The greater prevalence of *Fusarium* in Broadbalk as compared with Barnfield is probably related to the fact that wheat is a more favourable host for these forms than is the mangold, and the non-rotation of the wheat crop probably determines their increased presence. The scarcity of *Aspergillus* spp. and *Mucor* spp. in the Rothamsted cultivated fields was

unexpected but, nevertheless, the rarity of these two genera in the dilution plates was a definite and obvious fact throughout the year.

Further intensive work on the relative distribution of particular species and genera is needed before a satisfactory explanation of the presence or absence of the forms in particular kinds of crop fields can be found.

VI. SUMMARY

Quantitative

1. A direct correlation has been found between soil fertility as measured by crop growth and the number of fungi and actinomycetes in Barnfield (permanent mangold) and Broadbalk (permanent wheat) at the Rothamsted Experimental Station.

2. Inconclusive or negative evidence has been obtained with regard to the phenomenon of periodicity in the fungi and actinomycetes in the soil. Evidence, on the whole, seems to suggest that numbers throughout the year scatter about a mean value with an indication of lower numbers in the winter.

3. No marked and dominant effect of the crop has been observed upon the numbers of fungi and actinomycetes beyond a suggestion that the wheat field, on the whole, contains a greater number of actinomycetes than Barnfield while the reverse is the case for the fungi.

Qualitative

1. The results do not favour the view that there exist specific soil fungus floras determined by particular manurial treatments. The more fertile plots contain, however, a greater variety of soil fungi.

2. In the two fields different soil forms exist in different quantities. *Penicillium* and forms of *Dematium* are more prevalent in Barnfield while *Fusarium* tends to predominate in Broadbalk.

Forms of *Monilia* occur fairly uniformly in the two fields. Species of *Aspergillus* and *Mucor* are scarce in the two fields.

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EXPLANATION OF PLATES VI AND VII

Photographs of typical dilution plates, showing fungi and actinomycetes in the variously manured plots demonstrating the correlation between soil fertility and numbers of colonies.

PLATE VI. BROADBALK

	Treatment of plots	Plot no.
Fig. 1	Unmanured	3
Fig. 2	Sulphate of ammonia	10
Fig. 3	Sulphate of ammonia + superphosphate	11
Fig. 4	Sulphate of ammonia + superphosphate + sulphate of potash	13
Fig. 5	Dung	2 B

PLATE VII. BARNFIELD

Fig. 1	Unmanured	8 O
Fig. 2	Sulphate of ammonia	8 A
Fig. 3	Sulphate of ammonia + superphosphate	5 A
Fig. 4	Sulphate of ammonia + superphosphate + sulphate of potash	6 A
Fig. 5	Dung	1 O
Fig. 6	Dung + sulphate of ammonia	1 A

(Received 5 November 1935)

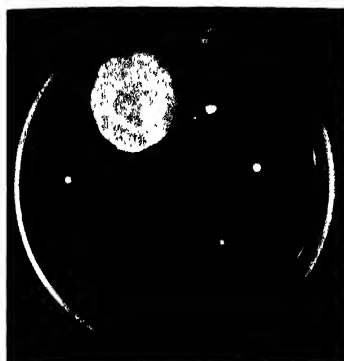


Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5

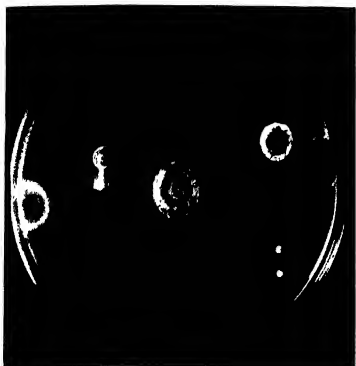


Fig. 1

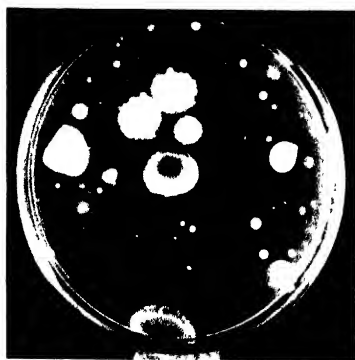


Fig. 2



Fig. 3

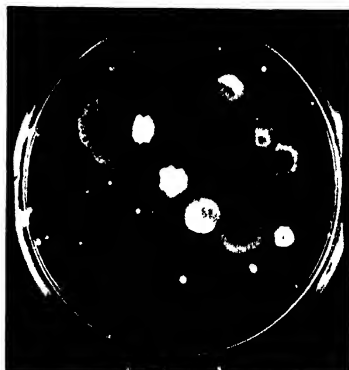


Fig. 4

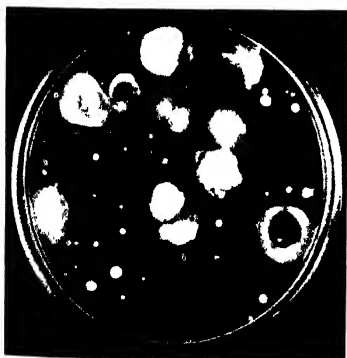


Fig. 5

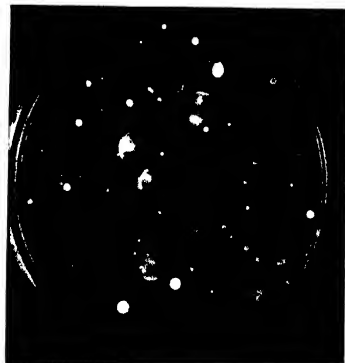


Fig. 6

SOME OBSERVATIONS ON THE OCCURRENCE OF *FUSARIUM CULMORUM* ON WHEAT

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IN published work dealing with the occurrence of *Fusarium culmorum* on cereals most attention has been devoted to the status of this fungus as a parasite. Simmonds(7), Bennett(1), Russell(5), Geach(3) and others have recorded it as causing seedling blight, foot rot, whiteheads or ear blight of oats and wheat in the field, and have proved its pathogenicity by controlled inoculation experiments. In recent years, however, references to its occurrence either as a weak secondary parasite, or under circumstances where its parasitic action has been doubtful, have been more numerous. One of the most interesting of these is the work of Broadfoot(2) in which isolations from many thousands of wheat plants taken at random from rotation plots yielded *F. culmorum* (either alone or in combination with other fungi and bacteria) to the extent of from 20 to 60 per cent in different plots. Simmonds(7) and Sanford & Broadfoot(6) have also isolated *F. culmorum* from stubble. The latter workers compared the virulence of many isolates and found that most of them were only weakly pathogenic. Apart from this recent work in Canada the information available on the weakly parasitic and saprophytic life of *F. culmorum* on cereals is comparatively scanty. It therefore seems worth while putting on record the results of isolations made from healthy wheat plants collected in England.

Isolations made at Rothamsted about harvest time in 1934 showed that *F. culmorum* was present on most of the roots and stem bases of wheat plants affected with whiteheads. However, control isolations made from healthy plants with full ears of grain revealed that *F. culmorum* was often present on the roots and stem bases of these plants also, so that no conclusion could be drawn as to the parasitic action of the fungus where it had been isolated from the whitehead plants.

In the following year, with a view to obtaining some idea of the progressive invasion of the roots and crowns of wheat plants by fungi, isolation work was begun about the time the crop was in flower, and periodic isolations were made as the crop ripened. The material for this study was obtained from three wheat fields, one at Rothamsted, one at St Albans and one at Cambridge (Table 1).

The crops on these fields were among the healthiest in the districts,

Table I. *Cropping data for fields examined*

	Soil	Variety	Sown	Reaped	Rotation	Yield per acre
Rothamsted	Clay with flints, pH 7.8	Victor	26 Oct. 1934	6 Aug. 1935	1932 kale 1933 barley 1934 beans	
St Albans	Clay with flints, pH 7.2	Victor	27 Sept. 1934		Temporary grass ley 1934 oats	50 bushels
Cambridge	Heavy gault clay, pH 7.9	Yeoman I	29 Oct. 1934	30 Jul. 1935	Temporary grass ley	58 bushels

and showed no evidence that seedling diseases had been present. At intervals of about a fortnight ten samples of about a dozen plants were taken from each crop. These samples were taken at intervals of ten paces and between ten and twenty paces in from the edge along the best side of the field. From each sample the healthiest plant was chosen, and four crown pieces and five root pieces cut out at random. These were rinsed in alcohol, sterilized two minutes in 1:1000 mercuric chloride, washed in sterile water, and plated out on potato-dextrose agar, the crown pieces and root pieces being plated in separate dishes. The last isolations were made from the stubbles after the crops had been cut. A summary of the results is given in Table II.

Table II. *Isolations of Fusarium culmorum and other fungi from healthy wheat plants*

Field	Date of collection	Part of plant	No. of pieces	Number of pieces yielding		
				<i>Fusarium culmorum</i>	other <i>Fusarium</i> spp.	other fungi and bacteria
Rothamsted	26 June	Crown	40	0	4	29
		Roots	50	5	2	36
	7 July	Crown	40	4	1	31
		Roots	50	2	2	41
	22 July	Crown	40	3	5	32
		Roots	50	2	3	45
	6 Aug. (stubble)	Crown	40	1	0	35
		Roots	50	0	1	46
St Albans	1 July	Crown	40	0	0	17
		Roots	50	1	0	15
	15 July	Crown	40	1	5	33
		Roots	50	5	3	36
	29 July	Crown	40	14	3	21
		Roots	50	10	2	33
	13 Aug. (stubble)	Crown	120	42	2	66
		Roots	150	34	0	106
Cambridge	12 July	Crown	40	13	0	23
		Roots	44	14	0	21
	26 July	Crown	40	11	4	25
		Roots	150	14	5	75
	20 Aug. (stubble)	Crown	120	90	0	4
		Roots	150	130	0	6

It will be seen that *F. culmorum* was isolated from a few specimens in every field at the time of the first sampling. In the Rothamsted field it did not increase in amount as the season advanced; in fact, it decreased. In the St Albans and Cambridge fields, on the other hand, it increased markedly, and in each, seven of the ten plants taken before harvest had the fungus on stem base or roots. In the field at St Albans thirty stubble samples taken at random over the field two weeks after harvest showed 70 per cent infection with *F. culmorum*, and in the Cambridge field the fungus was present on every one of fifty random stubble samples taken three weeks after harvest.

In spite of the appreciable amount of *F. culmorum* present on the roots at harvest time the crops gave excellent yields. The one at St Albans yielded 50 bushels to the acre, and the one at Cambridge 58 bushels. In the latter there were fourteen sacks of head corn and only two-thirds of a sack of tail corn, so that the grain had filled remarkably well. A sample of wheat from this crop took the Gold Cup at the Baker's Exhibition, London, for the best sample of milling flour.

To determine whether the strains of *F. culmorum* obtained were weak and almost non-pathogenic, isolates from the different localities were multiplied on corn-meal sand, and pathogenicity tests on wheat seedlings in the greenhouse were carried out in the usual manner. When the seedlings were washed out at the end of five weeks there was appreciable attack on the roots by all the strains of *F. culmorum* used. The average disease rating (4) for thirteen isolates of *F. culmorum* was 21.0 (ranging from 9 to 40), that for three isolates of *Ophiobolus graminis* was 99.0, and that for the controls was 7.5. Sanford & Broadfoot(6) have drawn attention to the difficulties encountered in greenhouse tests for pathogenicity when done by the usual methods. Not much reliance was therefore placed on these tests, and the question of the relative pathogenicity of strains of *Fusarium culmorum* in England was left for more detailed investigation in the future.

Although isolation work was not commenced quite early enough, and was not done on a sufficient number of samples to give a complete picture of the activity of *F. culmorum* during the season, it is evident that the fungus must have been present in the soil from which these healthy wheat plants were taken, exerting no appreciable parasitic effect and entering the root systems, along with other fungi, only as the roots began to lose vitality after flowering of the crop. There was then a still further development of the fungus on the stubble, after the crop had been cut. This is in contrast to fairly numerous cases reported, especially from the north of England, of appreciable damage to wheat by the fungus *F. culmorum*. The soil or other factors which favour parasitic action by this fungus during the earlier stages in the growth of cereal plants are at present very imperfectly understood, but

it may be significant that these three instances in which no apparent injury was done were all on slightly alkaline clay soils.

The assistance of Miss E. Holmes and of Dr M. Fernando with portions of the cultural work, and of Dr W. A. R. Dillon Weston in the collection of samples from Cambridge, is gratefully acknowledged. Dr F. J. Greaney shared in the work described in the paper while he was a temporary worker at the Rothamsted Experimental Station. He is a member of the staff of the Dominion Rust Research Laboratory, Winnipeg, Canada.

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THE SUSCEPTIBILITY OF THE PLANT CELL TO VIRUS DISEASE

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(With 1 Text-figure)

IN connexion with some micromanipulative work which had been planned it became necessary to find a technique whereby the presence or absence of virus in a small amount of the contents of a cell of an infected plant could be demonstrated. This need led to several experiments bearing on the susceptibility of the host-plant cell, attempts being made to discover a plant, which, if suitably inoculated, would serve as an index for such minute amounts of virus. Unfortunately no plant and inoculation method yielded more than one-tenth of the expected number of infections. This could only be explained by a variation in the susceptibility of the cells of the host plants. It was decided to abandon these methods and to seek a serological test for such small quantities of virus. Nevertheless, the results of the experiments, although they were not statistically planned and data in some cases are scanty, are of sufficient interest to be worth placing briefly on record.

INOCULATION WITHOUT INJURY TO THE CELLS OF THE HOST

Experiments were first made to determine whether infection can occur when all the cells of the parts of a plant coming in contact with the virus are quite uninjured.

In the first series, three host plants which show systemic infection with the yellow strain of aucuba mosaic disease (2) were used. Juice was extracted from infected tomatoes, passed through fuller's earth and diluted with water to 1 part in 3. The upper and lower surfaces of healthy plants were sprayed with this juice through a de Vilbiss atomizer. Great care was taken to avoid touching the sprayed leaves in any way. In the control plants the upper surface of one leaf was similarly sprayed and was then rubbed with the finger.

The results accruing are shown in Table I.

Table I

Effect of spraying uninjured plants with suspension of aucuba mosaic virus

Species	Results			Controls		
	No. treated	+	-	No. treated	+	-
<i>Solanum nodiflorum</i>	36	0	36	12	12	0
<i>Solanum lycopersicum</i>	96	2*	94	24	24	0
<i>Nicotiana tabacum</i>	24	0	24	9	9	0

* Plants showed insect damage.

In a second series of experiments *Nicotiana glutinosa*, which develops local necrotic lesions on infection with tobacco or aucuba mosaic virus, was used. Whole leaves were sprayed with infectious juice. After spraying, one-half of each leaf was left untouched; as a control, the other half was rubbed with the finger to break the hairs which project from the leaf surface. Some leaves were treated on the plant; other leaves were detached from the plant and were minutely examined for any possible damage before spraying. After treatment the latter were stored in Petri dishes on damp filter paper. Care was taken to handle the leaves only by their petioles. Table II gives details of these experiments.

Table II

Effect of spraying uninjured leaves of Nicotiana glutinosa with a virus suspension

Inoculum	Position of leaf	No. of leaves treated	No. of lesions on sprayed halves	No. of lesions on halves sprayed and rubbed
Aucuba mosaic (yellow strain) of tomato, dilution: 1 in 100	On plant	14	3*	1167
Aucuba mosaic (yellow strain) of tomato, dilution: 1 in 100	Detached	15	0	1685
Tobacco mosaic (Johnson's No. 1), dilution: 1 in 10	On plant	18	5*	1417

* Showed insect damage.

Tables I and II show quite conclusively that the virus is unable to enter uninjured cells. In the very few cases where infection resulted after spraying, the leaves had been damaged by insects, presumably whilst the virus suspension remained on the leaf surface.

These results are in accordance with those of Caldwell⁽¹⁾, who holds that the virus cannot enter an unbroken cell. He states that, even when the virus is contained in the xylem vessels of the host, it is necessary to crush the tissues to enable it to pass from the xylem into the living cells.

Duggar & Johnson(3), however, state that it is possible to infect tobacco plants by spraying the leaves with infective juice, the virus passing into the host through the stomata. It is suggested that, in their experiments, "gently lifting the leaf with the nozzle of the atomizer and spraying while the leaf was so held" caused sufficient damage to the delicate hairs clothing the tobacco leaf for the virus to gain entry through a broken cell.

PERIOD WHICH MAY ELAPSE BETWEEN INJURY TO THE CELL
AND THE ENTRY OF VIRUS

One-half of the adaxial surface of each of ten leaves of *Nicotiana glutinosa* was rubbed gently with the finger and immediately sprayed with a suspension of aucuba mosaic virus. The other half of each leaf was rubbed with the same virus suspension. The first method yielded 310 lesions; the second 329. It became evident that a high percentage of infection can be obtained if the virus is applied after injury is caused to the cells. Experiments were then made to determine how long after injury infection could still be brought about.

Leaves of *N. glutinosa* were rubbed gently with the index finger. One-half of each leaf so rubbed was immediately sprayed with a suspension of virus; the remaining halves were similarly sprayed after the lapse of a selected interval of time. In the first set of experiments intervals of 5, 10, 20 and 30 min. were selected. As it seemed desirable to obtain information regarding shorter periods, in the later experiments half-leaves were sprayed at intervals of 1, 2, 4, 8 and 16 min. after rubbing. Table III gives the results of these experiments, which are also graphically illustrated by Fig. 1.

Table III

Possibility of time elapse between injury to cell and entry of virus

Time interval in min.	No. of replicates	No. of lesions after interval	No. of lesions on controls	Lesions after delayed spraying Lesions after immediate spraying %
1	13	356	451	78.9
2	13	315	442	71.9
4	13	166	387	42.9
5	4	83	188	44.2
8	10	114	302	37.7
10	3	44	176	25.6
16	2	21	79	26.5
20	3	8	187	4.3
30	3	5	176	2.8

The results obtained do not accord with those of Holmes(4), who suggested that with *N. rustica* "rubbing is effective as an inoculation method only in the presence of virus", and that "wounds made

immediately before the application of virus were ineffective". Fig. 1 shows that although the chances of infection fall rapidly in the first few minutes after injury, an adequate dose of virus may still gain entry into the cell even half an hour after rubbing.

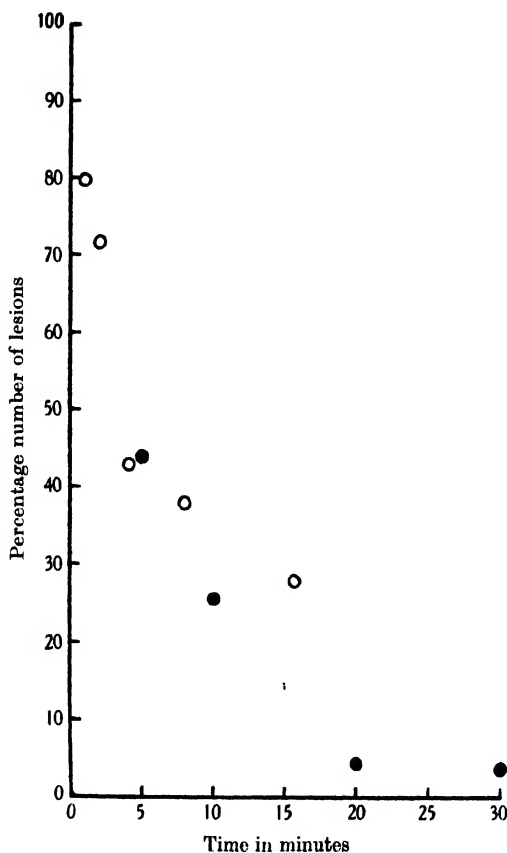


Fig. 1. The possibility of time elapse between injury to the cell and the entry of virus.

INOCULATION BY INJECTION INTO SINGLE CELLS

Doses of filtered but undiluted virus juice which should be adequate to cause infection were inoculated into single cells of growing plants.

Glass micropipettes of apertures $1-5\mu$ were drawn out and suitably bent. A micropipette was fixed into the manipulating stand of a Janse and Péterfi micromanipulator bearing a microscope. A second microscope was placed beside the micromanipulator on the same side as the stand

bearing the pipette and in such a position that the tip of the latter could be swung around, by adjusting the manipulating stand, from the optical axis of one microscope to that of the other. The second microscope was equipped with a glass stage on which was rested the leaf to be inoculated. To the objective of this microscope was attached a Busch diagonal illuminator. It was possible to pick up in the pipette a drop of virus juice from the cover-glass of the moist chamber and to inoculate it immediately into a cell of the leaf arranged beneath the second microscope.

Inoculations for systemic infection

Young plants of *Solanum nodiflorum*, tomato, tobacco and *Hyoscyamus niger* were inoculated with aucuba mosaic virus (yellow strain). One injection was made into each plant into the cells of either hairs, palisade or phloem tissues. No significant difference was observable between the results obtained by injection into different tissues. In all, 102 injections were made, nine infections resulting, i.e. a little less than 9 per cent. of the injections were effective.

Inoculations for necrotic lesions

Aucuba mosaic virus (yellow strain) was injected into the hairs or palisade cells of the leaves of *Nicotiana glutinosa*. The leaves were either detached from the plants and, after infection, stored in Petri dishes, or they were left on the plants which were returned to the glasshouse after inoculation. Leaves of that stage of development which experience suggested would give the greatest number of lesions were selected. Again the percentage of infection was low, and no significant difference was found between the percentage of infections resulting from injection into the hairs or into the palisade tissue. Of a total of 810 injections, 85 or 10.5 per cent. caused necrotic lesions to form on the leaves.

Similar injections were made into the cotyledons of the runner bean. Of these inoculations 15 in 240 or 6 per cent. resulted in the production of lesions.

Inoculations for starch lesions

The viruses of tobacco mosaic, aucuba mosaic and Hy. III diseases were injected into cells of hairs, palisade or phloem of tobacco leaves. A number of injections (usually fifteen) were made into one-half of each leaf; on the other half a standard dilution of virus juice was rubbed. The plants were returned to the glasshouse where they were left for a number of days varying according to the virus used and to the weather

conditions. They were then put in total darkness and kept there from 6 p.m. to 9.30 a.m., when leaves were cut from the plant and immediately killed by immersion in boiling water. The leaves were decolorized with alcohol and stained in iodine solution and the numbers of lesions were counted.

The results obtained are summarized in Table IV.

Table IV

Inoculation of virus by micropipette into tissues of the tobacco plant

Tissue injected	Virus	No. of replicates	No. of starch lesions	Per-centage of in-fections	Dilution of standard	Av. no. of lesions pro-duced by standard
Hairs	Tobacco mosaic	42	6	14.3	1 in 100	115
Palisade	" "	78	11	14.1	1 in 100	151
Hairs	Aucuba mosaic of tomato	162	8	4.9	1 in 10,000	26.4
Palisade	" "	57	7	12.3	1 in 10,000	57.5
Phloem	" "	105	19	17.1	1 in 10,000	75
"	Hy. III	240	16	6.6	1 in 100	58.5

When the results were analysed, the figures obtained from the standard proved of little value. Dr M. A. Watson,¹ working in this laboratory, has similarly found the half-leaf method to be of little value when more than two variants are to be considered, and Youden & Beale⁽⁵⁾ advocate the method when comparing only two samples of virus.

With tobacco mosaic no difference was found between the percentage of infections resulting from injection into the cells of the hairs and those of the palisade, but, with aucuba mosaic, inoculation into the palisade resulted in a higher percentage of infection. However, the χ^2 test shows this percentage not to be significantly greater. With aucuba mosaic, the percentage of infections obtained by inoculation into the phloem is found to be significantly greater than that obtained by inoculation of the hairs. Hy. III disease is normally transmitted by *Myzus persicae*, which is generally supposed to feed from the phloem of the host. For this reason, injections of Hy. III were confined to the phloem. The greater infection obtained by injection of aucuba mosaic than by Hy. III into the phloem may be explained by the greater infectivity of aucuba mosaic disease.

Inoculations by injection into single cells of various tissues of different hosts all yield low percentages of infection. This is not necessarily in disagreement with the results obtained by the more usual mechanical methods of inoculation. If, for instance, a leaf is rubbed with the finger

¹ Unpublished data.

or a cloth or spatula moistened with a virus suspension, a large number of hairs are torn or broken. To produce a systemic infection the virus needs to gain effective entrance through one only of these broken cells. If necrotic or starch lesions are shown, then the virus must enter the leaf at a number of cells. But the number of lesions produced is always exceedingly small as compared with the number of hairs on the leaf surface, and a considerable proportion of these may be broken by rubbing. The results accord with those of Dr M. A. Watson,¹ who finds that Hy. III virus is effectively carried by the aphid in only a small proportion of cases.

These low percentages of infection suggest that differences exist in the susceptibilities of cells to virus attack, not only in different leaves, but also in the same type of tissue and in the same leaf. It has been suggested that the varying susceptibilities of different leaves is due to some mechanical difference making tearing of the cells more difficult, and, for this reason, various abrasive substances have been mixed with the virus suspension. The fact that micropipette injections and infection by aphids yield low percentages of infection suggests that the varying susceptibility may be due to variation in some physical or chemical property rendering the cell contents antagonistic to the virus.

SUMMARY

A number of spraying experiments showed that the virus cannot enter a plant unless some of the cells are injured. It is not essential that such injury should be brought about in the presence of the virus. The chances of infection fall off rapidly in the first few minutes after injury, but infection occurs occasionally as long as half an hour after the cell is damaged.

Inoculations by micropipette into single cells of the host plant yielded only about one-tenth of the expected number of infections. This suggests differences in the susceptibility of the cells to virus attack.

¹ Unpublished data.

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THE ROLE OF PLASMODESMS IN THE TRANSLOCATION OF VIRUS

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(With Plate XXI)

THE literature bearing on the movement of the virus within the plant has been summarized by Henderson Smith⁽³⁾ and Kenneth Smith⁽⁴⁾. The balance of the evidence suggests that virus is not carried in the transpiration stream, and the suggestion has been made that it travels in the phloem. That virus does travel more rapidly in the region of the vascular bundles is shown by the curious deformations of the usually circular starch lesions when near the larger veins.

It is obvious that on occasions the virus must move about in the parenchymatous ground tissue of the leaf. In the case of systemic infection the virus appears to be carried into the primary meristem of the shoot and there multiplies as the cells become differentiated. Interference with the normal development of the plastids results in the production of mottled leaves⁽²⁾. But virus also finds its way into the older leaves which were fully developed when the plant was inoculated. If it is present in the palisade tissue of these leaves it must reach the cells by movement through ground tissue. Also when leaves are artificially inoculated by breaking of hairs in the presence of a virus suspension, the virus must then pass through the ground tissue. It has been suggested that the virus passes from cell to cell by "diffusion". Owing to the particulate nature of the etiological agent of these diseases, it is unlikely to pass from cell to cell by actual diffusion through the cell wall. It is suggested that it is carried along the protoplasmic strands which connect the cytoplasm of adjoining cells in the phloem and in the majority of other living tissues.

Over a period of years, the intracellular inclusion bodies induced by many viruses in their appropriate hosts have been examined. These inclusions occur to a greater or lesser extent in all tissues of the host. It was noticed that, although every cell over large areas of the epidermis

might contain inclusion bodies, the guard cells of the stomata even when included in these regions were invariably devoid of such bodies (Pl. XXI, fig. 1). It was therefore decided to examine the protoplasmic connexions between these cells.

Epidermal strippings were taken from leaves of tomato, tobacco, *Hyoscyamus niger* and *Solanum nodiflorum*, all plants which had been used extensively in the study of intracellular inclusions. These were treated by Crafts's technique for demonstrating plasmodesms⁽¹⁾. After fixing, the material was immersed in sulphuric acid and was then stained. The immersion in acid causes the walls to swell and the plasmodesms to become stretched as the protoplast contracts from the walls. The strength of acid used and the time of immersion were varied. A shorter immersion in a weaker acid revealed the protoplasts of adjoining cells connected together by protoplasmic bridges (Pl. XXI, figs. 2 and 3). A longer immersion or a stronger acid caused these bridges to break at either end, a fine strand of cytoplasm remaining within the cell wall (Pl. XXI, fig. 4).

Preparations so made in no case revealed any protoplasmic connexions between the guard cells of the stomata and the surrounding epidermal cells. That these guard cells contained no intracellular inclusions (Pl. XXI, fig. 1) suggests that, owing to the absence of any direct protoplasmic connexion with the adjacent cells, the virus is unable to pass into the guard cells.

That the virus travels through the plasmodesms is a possible explanation of the non-transference of virus to the offspring of diseased plants even in cases where fruits and seeds are infective. The growing embryo generally absorbs nutriment through specially developed haustoria which ramify between the cells of the maternal tissue. The detailed structure and performance of these haustoria varies from species to species, but usually there seems to be no protoplasmic connexion between the young embryo and the tissues of the parent. If the virus were present in the embryo prior to fertilization, it would be expected to multiply rapidly in the meristematic tissue of the developing embryo. If it were not present prior to fertilization, unless it were brought by the pollen tube, it would be unable to reach the embryo later.

Any evidence as to the movement of virus within the host must of necessity be circumstantial. The plasmodesms constitute an obvious route for the passage of particulate substances from cell to cell of the parenchymatous ground tissue of the plant. That no evidence of the presence of virus could be found in cells having no protoplasmic

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connexion with virus-containing cells, suggests that the plasmodesms are indeed the path taken by the virus.

SUMMARY

Although intracellular inclusion bodies may occur in every cell over large areas of the epidermis, they have not been found in the guard cells of the stomata. No protoplasmic connexions could be shown to exist between the guard cells and the surrounding tissues. These findings suggest that, owing to the absence of plasmodesms, the virus is unable to reach the guard cells. Support is lent to the view that, when the virus moves in the ground tissue of the host, it is carried from cell to cell along the protoplasmic bridges.

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EXPLANATION OF PLATE XXI

Photomicrographs were taken with a Leitz "Makam" camera using a Leitz 6L objective and a Leitz 10 \times periplanatic ocular. Magnification 450 \times .

- Fig. 1. Epidermis of *Solanum nodiflorum* showing large intracellular inclusions. There are no inclusions visible in the guard cells of the stomata. (Fixed, Carnoy's fluid; stained, Feulgen and orange G.)
- Fig. 2. Epidermis of tomato. Plasmodesms are shown between adjacent epidermal cells, but not between the guard cells and the epidermis. (Crafts's technique.)
- Fig. 3. Epidermis of *Solanum nodiflorum*. Protoplasmic strands connect the cytoplasm of adjoining cells, but the guard cells are isolated. (Crafts's technique.)
- Fig. 4. Epidermis of *Solanum nodiflorum*. Protoplasmic strands are seen in the swollen walls. (Crafts's technique.)

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Fig. 1



Fig. 2



Fig. 3



Fig. 4

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THE HISTOLOGY OF THE NECROTIC LESIONS INDUCED BY VIRUS DISEASES

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(With Plates XXXIV and XXXV)

INTRODUCTION

MANY viruses produce widely differing symptoms in different hosts. Some, which generally result in a systemic infection may, in a few hosts, induce the production of local necrotic lesions. Tobacco mosaic disease and aucuba (or yellow) mosaic of tomato produce a local necrosis in *Nicotiana glutinosa* although cucumber mosaic and Hy. III disease cause mottling in this same host. To produce systemic infection, the virus spreads from the seat of inoculation throughout the tissues of the host and into the primary meristem where it interferes with the normal development of the plant tissues⁽¹⁾. In tobacco, some diseases (e.g. aucuba mosaic) may induce local necrotic lesions on the inoculated leaf but the disease later becomes systemic. When necrotic lesions are produced in *N. glutinosa* or in the bean the virus is confined to a relatively small number of cells surrounding the seat of inoculation. These cells die, necrosis becoming macroscopically visible about 2 days after inoculation. Such lesions have proved of considerable importance in this field of pathological research, as they provide a quantitative test for the viruses producing them. It seemed desirable, therefore, to discover the special peculiarities in the behaviour of the host which result in the isolation of the virus within local necrotic areas.

MATERIAL AND METHODS

Juice was extracted from macerated leaves of tomato or tobacco plants infected with aucuba mosaic disease. A dilution of one part of this crude juice in two parts of water was used to inoculate the leaves of *N. glutinosa* plants. The juice was rubbed with the index finger on to one-half of each leaf, care being taken to injure nothing but the hairs. The unrubbed halves of the leaves were used as controls. Generally,

the virus was rubbed on to the adaxial surface of the leaf. As the work developed, it became necessary to inoculate a few leaves by rubbing the lower surface. As controls, leaves were rubbed with healthy juice and others with water.

Small strips cut from each half of each leaf were later fixed. In the summer under good growing conditions when the lesions became macroscopically visible in less than 2 days, fixations were made at intervals of 12, 24, 48 and 72 hours after inoculation.

A large number of fixatives was tried and of these, Zenker's, Champy's, Regaud's and LaCour's fluids were selected for further work. Owing to the waxy nature of the leaves and to the hairs on the surface, difficulty was experienced in making the leaves sink into the fixing fluid. This was to some extent overcome by immersing them in Carnoy's fluid for about 1 min. prior to using the appropriate fixative. The fixing bottles were then placed under an exhaust pump until the material would sink in the fluid.

After fixing the material was washed in running water, dehydrated in ethyl alcohol, cleared in cedar wood oil and embedded in paraffin wax of melting-point 52°C. Sections were cut at a thickness of 6-15 μ and were variously stained.

DESCRIPTION

The normal leaf

In transverse section the healthy leaf of *N. glutinosa* (Pl. XXXIV, fig. 1) shows a single row of closely packed, elongated, thin-walled palisade cells towards the upper surface. Below this are several layers of parenchymatous cells forming a meshwork with large intercellular spaces. Each surface is bounded by a highly cuticularized epidermis. The majority of the stomata are on the lower surface of the leaf where they are in direct connexion with the large air spaces of the spongy parenchyma. The surface of the leaf is protected by hairs which appear to be more numerous on the upper than on the lower side. Each hair grows out from an enlarged epidermal cell and consists of about four elongated cells tapering towards the apex. The walls of the hairs are strongly cuticularized.

The development of the lesions

Under good growing conditions lesions become evident on the dorsal surface of the leaves about 48 hours after inoculation. Minute, slightly sunken, dried patches of epidermis are visible on the lower surface a few hours previously. The spots do not all develop simultaneously, their

numbers being found to increase over several days. At first, the lesions appear as small, colourless shiny patches. For several days they increase in size, at the same time becoming dry and assuming a brown coloration.

The first reaction to the virus may become microscopically visible after 12 hours but more usually no abnormality is apparent until 24 hours after inoculation. The first visible evidence of infection is the appearance of a strip of dark staining material between certain of the cell walls. Occasionally, this band may first appear between the palisade cells, spreading rapidly downwards from towards the upper to the lower side of the leaf. More frequently the band is first formed between the cells of the lower epidermis and those of the spongy parenchyma (Pl. XXXIV, figs. 2 and 4).

The virus enters a leaf through hairs which were broken by rubbing (8). However, lesions do not always develop immediately below a broken hair. Presumably the virus may travel a short distance across, as well as through the leaf, before any reaction is shown. The dark staining disc is at first apparent over only two or three cells, but it rapidly spreads until it covers a hundred or more (Pl. XXXIV, fig. 4), completely cutting off the epidermis from the spongy parenchyma.

At the same time as this band is forming, mitosis occurs in the cells immediately within it. All stages in nuclear division from the prophase to the presence of two daughter nuclei within one cell were found (Pl. XXXV, figs. 1-4). Karyokinesis appeared to be normal, but no evidence of cytokinesis was found. At the telophase, no cell-wall formation was seen, and binucleate cells are of fairly frequent occurrence in the areas which are becoming necrotic. Mitosis usually occurs in the cells immediately within the epidermis (Pl. XXXIV, figs. 2 and 4 and Pl. XXXV, figs. 1-4) but it is sometimes more deep seated (Pl. XXXIV, figs. 6 and 7).

The necrotic disc has now isolated the epidermis, and the cells, being cut off from their supplies of nutriment, are drying up. The band begins to branch upwards towards the upper surface of the leaf (Pl. XXXIV, figs. 5-7). It ramifies between the cells of the parenchyma, ultimately penetrating through to the upper surface. As each cell is isolated it dies and dries out. Finally the whole lesion becomes dry and consists essentially of a meshwork of the dark staining necrotic material (Pl. XXXV, fig. 7).

That the changes described are all due entirely to the interaction of the virus and the host is shown by the examination of the control

material. When leaves were rubbed with water or with healthy plant juice, the only abnormalities found were broken hairs, there was no stimulation to mitosis nor was there any tendency to the formation of the dark staining disc.

Experiments were made to determine the nature of the necrotic material. The peculiarity of its first appearance being usually towards the lower surface where there are large intercellular spaces and many stomata suggested that it might be a product of oxidation. Two experiments were therefore made.

Three leaves were taken on each of six plants. Half of each leaf was rubbed with virus extract in the usual way on the upper surface. The other half was rubbed on the lower surface with the same extract. Both sets of lesions developed simultaneously. It was evident that placing the virus near the seat of the first visible reaction in no way hastened that reaction. There was, however, a difference in the numbers of lesions. A total of 2872 developed on the halves rubbed on the upper side, whilst rubbing the lower side resulted in only 1526 spots. This is probably due to there being fewer hairs on the lower surface of the plant. It also refutes the suggestion(2) that the virus enters through the stomata. As there are more stomata on the lower than on the upper surface, if the virus entered through them, the greater number of lesions would be expected to develop on the ventral surface.

Virus extract was then rubbed over the whole dorsal surface of some leaves. Immediately vaseline was smeared over half of the lower surface of each inoculated leaf. No difference could be discerned in the development of the lesions on the two halves of the leaves. These experiments indicated that the larger supply of air available near the lower surface is not essential to the rapid formation of the necrotic material.

This material is yellow in colour and on casual examination appears to have an affinity for basic dyes. Critical examination of stained preparations shows it to consist of large numbers of slightly elongated minute particles embedded in a matrix (Pl. XXXV, figs. 5 and 6). The particles are basophilic but the matrix stains with acid dyes.

As the substance appears first within the cell wall, it was thought that it might be of a pectic nature. Microchemical tests with ruthenium red and methylene blue on hand sections of fresh material and on microtome sections of fixed material lent no support to this view, nor did the substance react to treatment with pectinase. It proved to be insoluble in strong concentrated acids and in caustic alkalis. No positive reactions could be obtained with any of the more common reagents such as

Millon's, Sudan III, chlorzinc iodide, phloroglucin, ferric chloride, resorcin blue, etc.

DISCUSSION

That all living cells are capable of division has for some time been realized (1, 4). After wounding, a cambium may be formed across the cells of the most varied tissues in a layer near the surface of the wound. Meristems are formed also in response to fungal or bacterial infection.

In *Nicotiana glutinosa*, cell division normally ceases when the leaf is less than 1 cm. in length. Cell differentiation then commences and further growth is due entirely to enlargement of the cells. The leaves at the time of inoculation are about 10 cm. long and cell division has ceased 2 or 3 weeks previously. At the time of inoculation the spongy parenchyma cells are large and vacuolate and the nuclei are relatively small. It is obvious that without some external stimulus, no further division would occur. The necessary stimulus is supplied by rubbing the surface of the leaves with virus. That the mitosis is not a wound reaction is shown by the examination of leaves rubbed with water or with juice extracted from healthy plants. Such leaves fail to reveal any nuclear divisions, suggesting that the stimulation is provided directly or indirectly by the virus. Possibly this unusual mitosis is an attempt by the host to isolate the etiological agent of the virus disease by hyperplastic growth, and the cells are overcome by necrosis before new growth can occur. Or, possibly, the virus causes chemical reactions in the cells which produce the necrotic material and the products of reaction stimulate the nuclei. It is known that small doses of toxic substances often cause excitation of the cell contents (7).

It seems most probable that the mitosis is directly due to stimulation set up by the virus. In a number of solanaceous hosts infected with systemic diseases it has been found that the first symptom of the virus to become microscopically visible is a stimulation of the cytoplasm (5, 7). In these cases nuclear division is not found. The woody disease of *Passiflora edulis* caused by a virus (1) results in abnormal formations through hypertrophy of the pericarp. In sugar beet infected with curly-top disease, cells adjacent to lesions are stimulated to growth and division (3). The occurrence of karyokinesis as the lesions develop in *Nicotiana glutinosa* seems to be a parallel case of direct response to the stimulus of the virus.

The necrotic band formed between the cells may be due to the increased and abnormal metabolism causing exudation of waste products

from the cells. The necrotic material may cause suffocation of the tissues within it by preventing free interchange of gases with the atmosphere and so preventing the continuation of cell division, or the necrotic materials may be toxic to the cells.

When the virus first enters the leaf it obviously multiplies and travels rapidly through the cells. The response of the host is even more rapid, free interchange of materials between the infected cells and the surrounding tissues is soon prevented by the necrotic barrier. The virus is isolated within a relatively small area of the leaf within about 48 hours after infection.

SUMMARY

About 12 hours after a leaf of *Nicotiana glutinosa* is inoculated with aucuba mosaic disease a band of necrotic material begins to form within the cell wall, usually between the lower epidermis and the spongy parenchyma. This band extends both laterally and towards the upper side of the leaf. At the same time, nuclear division is observed in the spongy parenchyma cells, but karyokinesis is not followed by cell division. As the necrotic meshwork extends the cells within it die and dry out. After about 3 days, the lesion consists of a meshwork of this necrotic material. The cells are all dead and the virus is isolated within the necrotic area, all interchange between the infected and healthy parts of the leaf having been prevented.

I am indebted to Fraulein Lina Cunow for assistance in making the preparations used in this study.

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EXPLANATION OF PLATES XXXIV AND XXXV

All photomicrographs were taken with a Leitz "Makam" camera. Leitz objectives 3 b or 6 L achromatic or 2 mm. apochromatic (N.A. 1.4) were used in combination with Leitz periplanatic oculars $\times 10$ or $\times 6$.

A Leitz "Monla" lamp, suitably screened with Wratten colour filters, was employed as the source of illumination.

The fixative and the stain used in making the preparation, together with the colour filter used to take the photograph, is given in brackets after the description of each figure.

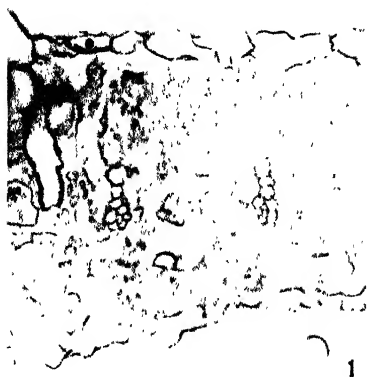
PLATE XXXIV

- Fig. 1. Transverse section of normal leaf of *Nicotiana glutinosa* showing single layer of palisade tissue and several layers of spongy parenchyma. The leaf is surrounded by a cuticularized epidermis. $\times 280$. (Lacour. Safranin-light green. Orange 22.)
- Fig. 2. 24 hours after inoculation. Transverse section of leaf showing early formation of necrotic material within the lower epidermis. Mitosis is occurring in a cell immediately within the necrotic band. $\times 280$. (Zenker. Crystal violet-erythrosin. Green 58.)
- Fig. 3. 24 hours after inoculation. Transverse section of leaf showing early formation of necrotic material. $\times 280$. (Zenker. Safranin-light green. Green 66 and blue 45 A.)
- Fig. 4. 48 hours after inoculation. The necrotic disc is extending laterally. Mitosis occurs in the cells immediately within the band. $\times 140$. (Zenker. Safranin-light green. 66 and 45 A.)
- Fig. 5. 48 hours after inoculation. The necrotic material extends upwards through the leaf. $\times 140$. (Zenker. Safranin-light green. 66 and 45 A.)
- Fig. 6. As fig. 5. Mitosis occurs in more deep-seated cells. $\times 450$. (Zenker. Safranin-light green. 66 and 45 A.)
- Fig. 7. 48 hours after inoculation. The necrotic material has spread almost to the upper surface of the leaf. Mitosis occurs in the upper epidermis. $\times 450$. (Zenker. Safranin-light green. 66 and 45 A.)

PLATE XXXV

- Fig. 1. 48 hours after inoculation. Metaphase in a cell within the necrotic disc. $\times 900$. (Zenker. Safranin-light green. 66 and 45 A.)
- Fig. 2. 48 hours after inoculation. Anaphase in a cell within the necrotic band. $\times 900$. (Zenker. Safranin-light green. 66 and 45 A.)
- Fig. 3. 48 hours after inoculation. Telophase in a cell within the necrotic band. $\times 900$. (Zenker. Safranin-light green. 22.)
- Fig. 4. 48 hours after inoculation. Two daughter nuclei within one cell within the necrotic disc. $\times 900$. (Zenker. Safranin-light green. 66 and 45 A.)
- Figs. 5 and 6. 48 hours after inoculation. High power photographs of the necrotic material show it to be of a particulate nature. $\times 900$. (Zenker. Crystal violet-erythrosin. 22.)
- Fig. 7. 72 hours after inoculation. Transverse section of leaf passing through a necrotic lesion. $\times 84$. (Zenker. Safranin-light green. 66 and 45 A.)

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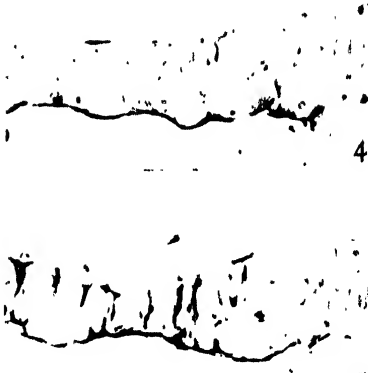
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The Isolation and some Properties of Liquid Crystalline Substances from Solanaceous Plants infected with Three Strains of Tobacco Mosaic Virus

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[Plates 12, 13]

Tobacco mosaic was the first disease shown to be caused by a filter-passing virus, and Beijerinck (1898) suggested as its cause a "contagium vivum fluidum". Since then many other theories have been advanced, but there has been little positive evidence to indicate whether the virus more nearly resembled organisms such as small bacteria or chemical molecules such as the larger proteins. Recently, however, Stanley has isolated from tobacco (1936*a*) and tomato (Stanley and Loring 1936) plants suffering from mosaic a protein which he describes as crystalline and as possessing the properties of the virus. When susceptible plants were inoculated with this protein at a dilution of 10^{-9} they developed typical symptoms of the disease. The protein was obtained from infective sap by repeated precipitation with 40% saturated ammonium sulphate solution, and by adsorption on and washing from celite. The "crystals" described by Stanley were small needles produced by precipitation with acid ammonium sulphate.

A number of statements in Stanley's earlier paper (1935*a*), more especially those dealing with the nitrogen content and the serological activity, made us doubt the purity of his product, and preliminary experiments with methods similar to those used for the preparation of suspensions of potato virus "X" (Bawden and Pirie 1936) gave us products with much higher precipitation end-points with antisera than those claimed by Stanley. We have now exchanged material with Dr. Stanley and find no gross differences

in the activities of our respective products. We have found, however, that by further purification the protein in neutral solution can be obtained in liquid crystalline states. Also, as will be shown later, there are considerable differences in the chemical descriptions given of the virus protein; some of these differences have already been resolved, and others presumably will be by future work.

VIRUS STRAINS USED

Tobacco mosaic virus is known to exist in a number of strains which possess similar properties *in vitro*, e.g. resistance to ageing, temperature, etc., yet differ from one another in the ease with which they can be transmitted to different hosts and in the type and severity of symptoms they cause in infected plants. We have worked with three such strains and have isolated liquid crystalline proteins from various plants infected with each. Irrespective of the host plant from which it was obtained, inoculation of the isolated protein to susceptible plants caused a disease characteristic of the particular virus strain used, and from such infected plants more of the same protein could be isolated.

The three strains used were a mild strain of the common tobacco mosaic virus, aucuba mosaic virus and Enation mosaic virus. The first was derived from a single local lesion on *Nicotiana glutinosa* produced by inoculating with diluted sap from a plant infected with Johnson's tobacco virus 1. The second was kindly provided by Dr. F. M. L. Sheffield and the third by Dr. G. C. Ainsworth. The symptoms produced by each strain vary with the age and conditions under which the host plants are grown. Those briefly described below are characteristic of plants growing under good light conditions in an insect-proof glasshouse at an average temperature of 70° F.

The strain of tobacco mosaic virus used causes in tobacco (vars. White Burley and Turkish) a preliminary clearing of the veins, followed by a general green-type mottling, with a few darker green blotches around the veins and occasional small yellow flecks. In tomato (var. Kondine red) it causes a general faint mottle and no deformation of the foliage; in old plants symptoms are often completely masked, so that the hosts behave as carriers.

Symptoms of aucuba mosaic have been described in detail by Henderson Smith (1928). In tomato and *Solanum nodiflorum*, the hosts used most in this work, they consist of a brilliant yellow mottle. Tobacco also was used but was not a convenient host because of the difficulty in getting uniform

results. On large tobacco plants no systemic infection was obtained: the inoculated leaves developed severe necrotic local lesions, but the uninoculated leaves remained healthy. Systemic infections were usually obtained in small seedlings, and these showed a bright yellow mottle, but they were so crippled by the disease that they yielded only small amounts of sap.

The hosts used for Enation mosaic virus were tobacco and tomato. Inoculated tobacco plants developed severe necrotic local lesions similar to those caused by aucuba mosaic virus, and, unless the plants were small, usually did not all become systemically infected. Those that did become infected showed symptoms similar to, but rather more severe than, the symptoms of plants infected with tobacco mosaic virus. On tomato, however, the disease caused by this strain is quite characteristic. Infected tomato plants developed a general mottle. The leaves were severely deformed and much dwarfed, often consisting of little more than the main veins, so that the picture obtained was typical "Fern-leaf". The under-surfaces of the leaves frequently developed leafy outgrowths, the so-called enations, which vary in size from minute ridges to large leafy frills (Ainsworth 1935).

Although the symptoms produced by the three virus strains have remained reasonably constant, it is not thought that each source necessarily contains only one strain. Indeed, it is more probable either that the sources are not pure or that they are continually mutating, for, from the yellow flecks appearing in the tobacco mosaic plants, material for an inoculum could be obtained that caused a general, bright yellow mottle in tobacco. This phenomenon has been fully described by Jensen (1933).

All three virus strains produce necrotic local lesions in *Nicotiana glutinosa*. They are serologically related, and all three are neutralized *in vitro* by, and precipitate with, the serum of rabbits immunized against the sap of plants infected with tobacco mosaic virus. Further, tomato plants infected with tobacco mosaic virus were found to be immune to further infection with either aucuba mosaic or Enation mosaic viruses.

ACTIVITY OF THE PURIFIED VIRUS PREPARATIONS

Liquid crystalline proteins have been isolated from all the plants affected with the diseases described, and the kind of protein isolated was found to depend entirely on the virus strain and not at all on the host plant used. The activity of the proteins was tested in two ways: (1) for their infectivity, and (2) for their precipitation end-point with the serum

of rabbits immunized by a course of intraperitoneal injections with crude sap from plants infected with the tobacco mosaic virus. As with potato virus "X" preparations, the two methods of measuring activity agreed quantitatively only when the preparations which were being compared had been treated similarly before they were tested (Bawden 1935).

Infectivity tests were made by the local-lesion method. At first both *N. glutinosa* and Golden Cluster beans were used, but as under the glass-house conditions at Rothamsted the beans proved both less sensitive and more variable, in later work only *N. glutinosa* was used. The number of local lesions produced by the same purified virus preparation varied considerably with the age and condition of the host plant, and with the position of the inoculated leaves on the stem. In determining the effect of dilution in individual experiments the host variation was reduced to a minimum by arranging the inoculations in the form of Latin squares. Each dilution was inoculated once to each plant and once to a leaf occupying the same relative position on the stem. When two virus preparations were being compared over a range of dilutions the Latin square was split for half-leaf comparisons, solutions of the same concentration being inoculated to opposite halves of the same leaves. The inoculations were made with ground-glass spatulae, and the fluids were rubbed as evenly as possible over the leaves. In the infectivity tests dilutions were made in 0.1 M phosphate buffer at pH 7, and in the serological tests in 0.85 NaCl solution.

The precipitation end-point determinations were made in 7 mm. thick-walled glass tubes, 1.0 c.c. of antiserum at a dilution of 1 in 50 being added to a series of tubes each containing 1.0 c.c. of protein solution at various concentrations. The tubes were immediately placed in a water-bath at 50° C. with fluid columns half-immersed to ensure complete mixing by convection currents. Readings were made after 24 hours, the first twelve being at 50° C. and the second at room temperature. The end-point was taken as the amount of protein in the tube containing the smallest precipitate visible to the eye.

The results of a few such tests on purified virus preparations before and after drying over P_2O_5 are given in Table I.

The infectivity of our preparations is of the same order as that obtained by Stanley (1936a) with tobacco mosaic virus, but the serological activity is from 50 to 100 times as great, for Stanley obtained precipitates with $1/10^5$ g. but not with $1/2 \times 10^5$ g. In a recent paper on aucuba mosaic virus (1937) Stanley states that "solutions containing slightly less than $1/10^6$ g./c.c. may give the precipitin test...but that at such high dilutions the determinations are not reproducible at will". Stanley does not give

the methods used in his tests, and it is therefore difficult to account for these differences. Using the method described above, the serological titres of from $1/10^6$ to $1/10^7$ with our preparations are readily reproducible. With the more highly purified virus preparations to be described later precipitation end-points of $1/10^7$ are common, and repeated tests on the same preparation give quite constant results. The serological titre is a reliable index of the antigen content of a solution, for not only are the results obtained reproducible but the end-point is relatively independent of the concentration of antiserum used. All the tests reported in this paper were

TABLE I—ACTIVITY OF THE PURIFIED VIRUS PREPARATIONS,
BEFORE AND AFTER DRYING OVER P_2O_5

Virus	Serological titre	Infectivity on <i>N. glutinosa</i> Average no. of lesions per half-leaf at various dilutions						
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Tobacco mosaic undried	$1/6 \times 10^6$	125	44	19	2.4	1.3	0.14	0
Tobacco mosaic dried	$1/2 \times 10^6$	103	22	9	1.5	0.3	0.14	0
Tobacco mosaic undried	$1/8 \times 10^6$	—	90	37	6	2	0.4	0.2
Tobacco mosaic dried	$1/4 \times 10^6$	—	56	29	3	0.3	0.14	0
Tobacco mosaic undried	$1/8 \times 10^6$	208	120	28	3	1	0.25	0.125
Aucuba mosaic undried	$1/10^7$	—	71	15	3.5	0.5	0.16	0
Aucuba mosaic dried	$1/4 \times 10^6$	—	42	6	1.3	0.16	0.16	0
Aucuba mosaic undried	$1/10^7$	105	70	17	3	0.2	0	—
Enation mosaic undried	$1/6 \times 10^6$	176	76	13	1.33	0.5	0.16	—
Enation mosaic dried	$1/2 \times 10^6$	104	47	9	0.66	0.66	0	—
Enation mosaic undried	$1/5 \times 10^6$	149	36	4	0.6	0.2	—	—

Footnote to Table I. The serological titre is the number of grams in 1 c.c. of a solution that gives a visible precipitate after 24 hr. with 1 c.c. of antiserum at 1 in 50. Dilutions in the infectivity tests are given as grams per c.c. The infectivity tests were arranged in the form of split Latin squares with from six to nine replications, i.e. 1 c.c. at each concentration was rubbed over from six to nine half-leaves.

made with antiserum at a dilution of 1 : 50, but deviations from this figure affect only the position of the optimal precipitation point and have no appreciable effect on the precipitation end-point of a virus preparation.

The infectivity tests, however, give much less constant results. The number of local lesions obtained depends largely on the host plant, and can be altered by varying the conditions under which the plant is grown. Repeated tests on the same virus preparation have given infection end-points varying from $1/10^8$ to $1/10^{11}$. The relationship between dilution and infectivity is rarely a linear one, but the range over which the fall in the

number of lesions is most nearly proportional to dilution is from $1/10^5$ to $1/10^8$.

No large differences between the activities of preparations of the different virus strains have been noticed, but accurate comparative infectivity experiments have not been made. All the precipitation end-point determinations were made with antiserum to tobacco mosaic virus, and with this the preparations of Enation mosaic virus have given slightly lower serological titres than have those of the other two strains.

Purified preparations of all three strains are antigenic, and antisera have been prepared against each. Each virus precipitates with and is neutralized *in vitro* by all three antisera. From the results of preliminary cross-absorption experiments, however, it seems probable that although the three are serologically related they are not antigenically identical, and that each contains specific as well as common antigens.

It is still not proved that the liquid crystalline proteins, which we shall describe, are the viruses, for it is not possible to state that our preparations contain only particles capable of infecting susceptible plants. The infectivity results show that solutions must contain about 10^{-9} g. of protein per c.c. to cause infection by the methods used. This weight of protein would represent a large number of virus particles. As the amount of virus required to produce a given number of local lesions varies with individual plants (and with the conditions under which they are grown) it is possible that a natural resistance of the plant has to be overcome before infection can occur, and a large number of particles may be necessary to do this. Also, the method of inoculation is relatively crude and there must be a large wastage of inoculum on the leaf surface. However, until infection can be obtained with much less protein, we are unable to see how conclusive evidence that the protein is virus can be obtained.

The serological results show that our preparations have precipitation end-points similar to those given by the more highly purified antigens; from the serologist's point of view, therefore, they may perhaps be regarded as relatively homogeneous. The purified virus preparations do not precipitate with the serum of rabbits immunized against either healthy tobacco sap or against sap of plants infected with viruses not related to tobacco mosaic virus. There is a great deal of circumstantial evidence indicating that the extra antigens in virus-infected plants may be the viruses themselves, but this again is not proved. There is a possibility that the antigens we have isolated are not the viruses but abnormal proteins produced as a result of the disease and contaminated with small amounts of virus. We consider this improbable, but there is no conclusive evidence

against it. With potato virus "X" it has already been shown (Bawden 1935; Bawden, Pirie and Spooner 1936) that antigenicity can be dissociated from infectivity by chemical treatment, and Stanley (1936*b*) recently obtained similar results with tobacco mosaic virus. We have found with our preparations of tobacco mosaic viruses that severe treatments such as heat, strong acid or alkali destroy their infectivity, antigenicity and crystallinity, whereas less severe treatments, such as nitrous acid or subjection to X-rays, may destroy infectivity without affecting antigenicity or crystallinity.

The data from centrifuging experiments and X-ray measurements, which will be described later, suggest that our preparations consist solely of particles that are very similar to one another in size and shape. The chemical evidence shows that successive isolations of protein agree with each other closely, and that the chemical properties are not changed by further fractionation. It is reasonable to assume, therefore, that if all the particles in our preparations are not virus particles capable of infecting plants, they are at least similar to virus particles in their superficial properties. We have no evidence that virus activity can be dissociated from the isolated proteins, and for simplicity shall refer to them as virus. *We shall assume, however, that the reservations stated above are remembered when, in the remainder of this paper, we speak of our product as virus.*

PREPARATION

Infected leaves are minced in a meat mincer and the sap expressed in a hand press. The sap can usually be clarified by centrifuging immediately, but sometimes it clears more easily if allowed to stand overnight or if it is first adjusted to about pH 5.2. Alternatively, it may be clarified by filtration through celite or Kieselguhr. The procedure we adopt for the isolation varies somewhat according to the state of the sap, but the method given in detail below can be used successfully with all types of sap we have yet encountered. With sap from young plants which have not long been infected, however, it is possible to shorten the process considerably.

One volume of alcohol is added to the clarified sap, and the greyish brown precipitate is centrifuged off immediately. The precipitate contains all the virus: there is evidence, which we shall present later, that this precipitation leads to an irreversible aggregation of the virus particles. The final product, therefore, is not strictly in the same state as the virus in the plant sap. This aggregation, however, is at present unavoidable, for

a similar aggregation follows precipitation with acid and with ammonium sulphate, and we have no other effective means of isolation.

Attempts to isolate the virus by high-speed centrifugation or by filtration on collodion membranes have not as yet given products with a serological activity comparable with that of the preparations described below.

The alcohol precipitate is suspended evenly in from five to six times its volume of water and again centrifuged. The first extract contains little or no virus and is discarded. The extraction of the precipitate is now repeated until a sample of the extracts no longer gives a precipitate with phthalate buffer at *pH* 3.3. From six to eight washings are usually necessary to remove all the virus from the chocolate-coloured residue. The collected supernatant fluids are now brought to about *pH* 3.3 by the addition of *N*/10 HCl. A white precipitate with a very characteristic satin-like sheen is produced, and this is centrifuged off. This precipitate is now suspended in water and dilute NaOH added to bring the *pH* to 7, when the fluid is again centrifuged until clear. The darkly coloured precipitate is discarded, and a third of a volume of saturated ammonium sulphate solution is added to the supernatant. This again produces a precipitate with a sheen and, after centrifuging, a yellow-coloured supernatant liquid which is discarded. The precipitate is taken up in water and again precipitated by adding a third of a volume of saturated ammonium sulphate solution.

The precipitate is now dissolved in water, using about 50 c.c. for the amount obtained from a litre of sap, and *N*/10 HCl is added to bring the *pH* to 3.3 (measured with bromophenol blue). This usually requires about 4–5 c.c. of acid. The precipitate is centrifuged off, resuspended in water and again centrifuged. The precipitate is now dissolved in *N*/50 NaOH, diluted to 50 c.c. and centrifuged if not quite clear. The neutral solution is now again brought to *pH* 3.3 and the precipitate centrifuged off. The precipitate is now relatively free from salts, and in this condition the material is soluble at *pH* 3.3, i.e. in *N*/200 HCl. 0.5 c.c. of *N*/20 HCl is therefore added to the precipitate and the mixture is stirred until it is homogeneous. When diluted to about 50 c.c. with water, a clear or slightly opalescent solution is obtained from which a little impurity can usually be removed by centrifuging. The virus itself, giving the characteristic satin-like sheen, can now be precipitated from the solution merely by adding 0.5 c.c. of 10% NaCl solution. Alternatively, the virus can be precipitated from the salt-free solution by adding enough dilute NaOH to bring the *pH* to about 4. The precipitate obtained in this way, however, seems to be amorphous.

The salt-free precipitate is then dissolved in enough dilute NaOH to

give a solution at about pH 6.5. Such a solution remains active for months when kept cold. If kept at room temperature it should be protected from evaporation, but it is unnecessary to adopt strict sterile precautions for periods of a few days, as bacteria and moulds do not grow at all readily in these solutions.

Solutions prepared in this way should be nearly colourless and slightly opalescent. Preparations of the three different strains show no consistent differences, although different batches of the same virus strain may vary considerably in appearance if they contain different amounts of impurities. The preparations, if not weaker than about 4 %, should separate into two layers after standing for some time. Each layer is liquid and contains virus, but the lower is both a stronger solution and also contains less impurity than the upper. This phenomenon and its application to further purification will be more fully described later.

Some preparations at this stage are still coloured, and on standing will not separate into layers. If the pigmentation is noticed early on in the preparation, or on a preliminary sample, a great improvement can be made by repeating the precipitation with alcohol on the eluate from the first alcohol precipitate. If, however, the virus has already been precipitated with acid it can no longer be precipitated with 50 % alcohol, for this precipitation depends on the presence in the fluid of certain other plant constituents, particularly calcium salts. We have sometimes further fractionated an impure sample of virus by precipitation with 50 % alcohol in the presence of added calcium chloride, but after such treatment it is often difficult to get the virus back into solution.

Coloured virus preparations that will not layer can, however, always be further purified by incubation with trypsin. Solutions containing about 0.5–2 % of solids and 0.2 % of commercial trypsin with a drop of chloroform added as a disinfectant have been incubated at 37° C. and at pH 8 for from 10 to 30 hr. This treatment has given invariably a product that becomes colourless after two to three further precipitations with acid and dilute ammonium sulphate solution. No differences have been noticed between material purified with trypsin and that prepared without, but the possibility that after treatment with trypsin the final product may be a complex cannot be excluded. The difference in size between the virus particle and the trypsin molecule is, however, so great that the combination of one molecule of the enzyme to one particle of the virus might well have little effect on the physical properties of the virus.

The yield of purified virus obtained has varied from 1 to 2 g/l. of expressed sap. The yield is greater from plants grown in the summer, and is

greatest from young actively growing plants that have been infected for about 3 weeks to a month. From such plants also it is easier to isolate the virus, and all the steps described above are not necessary. The sap from young tobacco and tomato plants is not highly pigmented and the treatment with alcohol can be omitted, the virus being precipitated directly from the clarified sap with either acid or ammonium sulphate. The brown material (largely carbohydrate), which in the previous method is rendered insoluble by the alcohol, can now be removed by treatment with ammonium sulphate. Some is not precipitated with a quarter saturation, and some does not dissolve after precipitation. The ammonium sulphate precipitate, therefore, should be suspended in about two to three times its volume of water and centrifuged until only slightly opalescent in transmitted light. By reflected light the supernatant fluid should have an intense sheen. The virus can now be freed from the ammonium sulphate by repeated precipitation with acid.

In recent preparations we have found that a considerable saving can be effected by heating the crude infective sap to 70° C. before clarifying it. This produces a precipitate that aggregates quickly and can be removed by a few minutes' centrifuging or by filtration. This treatment not only clarifies the sap much more readily than the other methods but it also gets rid of considerably more of the plant constituents. After heating, the virus can readily be isolated from the supernatant or filtrate by direct precipitation with acid or ammonium sulphate, and the treatment with alcohol can safely be omitted. The yield resulting from this method is good, and when large quantities of sap are handled it is more convenient than the other methods.

ANALYSIS OF THE PURIFIED VIRUS PREPARATIONS

Dried neutral solutions of the three virus strains have analytical compositions falling within the following range:

		%			%
Carbon	...	49.3-50	Phosphorus	...	0.45-0.55
Hydrogen	...	7.2-7.4	Ash	...	1.5-3.0
Nitrogen	...	14.4-16.6	Carbohydrate	...	2.5
Sulphur	...	0.24-0.59			

Part of the ash is produced by the sodium hydroxide used for the neutralization, but even after prolonged dialysis of the virus preparations at about pH 3.3 some ash remains. One gram of these proteins requires from 0.2 to 0.23 millimols of NaOH to take it from pH 3.3 to 6.5, and their

apparent equivalent weight is therefore about 4000. Phosphorus estimations were carried out by the Fiske and Subbarow method (1925) after incineration with sulphuric acid and perhydrol. This method was adopted after it was found that the Pregl method gave very unreliable results. The phosphorus content, however, has been confirmed by precipitation both as phosphomolybdate and as ammonium magnesium phosphate. The carbohydrate estimations were made by an orcin method (Pirie 1936) based on that of Tillmans and Philippi (1929). No consistent differences have been noticed between preparations of the three different virus strains.

When dried at pH 3.3 a preparation of Enation mosaic virus had the following composition: carbon 51.2 %, hydrogen 7.1 %, nitrogen 16.7 %, sulphur 0.26 %, phosphorus 0.51 % and ash 1 %. The figures for carbon, hydrogen and nitrogen fall within the range usual for proteins, and are similar to those published by Stanley (1936*a*) in his final paper on tobacco mosaic virus; then, however, he found neither phosphorus nor sulphur, and he makes no mention of carbohydrate. More recently (1937) he has agreed that his preparations of aucuba mosaic virus contain the amount of phosphorus and sulphur that we find, and it seems probable that further work will show that his tobacco mosaic virus preparations are very similar also. The sulphur contents of our preparations are variable. Although we have never had a preparation containing less than 0.2 % of sulphur it is possible that this may be an impurity, but it cannot be removed either by dialysis or by the prolonged centrifuging of a virus solution containing barium ions. Also, it follows the virus persistently through the various other purification processes that will be described.

The phosphorus and carbohydrate contents of our preparations are very constant. They are unaffected either by prolonged dialysis in cellophane tubes against dilute acid or alkali, or by reprecipitation ten times with either acid or quarter-saturated ammonium sulphate solution. From precipitates with their antisera, and with papain and clupein sulphate, the viruses have been recovered with their full activity and with their phosphorus content unaltered, and incubation with trypsin preparations rich in nuclease has no effect on the phosphorus content. All the treatments that we have tried which in no way inactivate the virus preparations leave the phosphorus content unaltered. Some treatments that do inactivate them, such as heating to 90° C. or exposure to strong acid or alkali, split off a nucleic acid or its breakdown products. Other treatments, however, that also inactivate them have no effect on the phosphorus content, e.g. nitrous acid, which destroys the infectivity without affecting the serological activity of the preparations, and drying, which affects both. We are

~~therefore~~ unable to agree with the statement of Stanley (1937) on ~~mosaic~~ *mosaic virus*, that the nucleic acid is merely a contaminant and that it is ~~incidental~~ to activity.

FURTHER PURIFICATION OF THE VIRUS PREPARATIONS

It has already been stated that solutions containing more than about 4% of the proteins, prepared as described above, after standing for some time will separate into two layers (fig. 1, Plate 12). The two layers have different solid contents and different appearances, both when viewed by ordinary light and through crossed Nicol prisms. The upper layer is the more dilute, and if separated from the lower and further concentrated, either by cautious evaporation in a vacuum or by precipitation with acid and resolution in a smaller volume of water, it will give more of the lower layer. Perhaps the simplest method of getting more lower layer is to freeze the upper-layer fluid in a centrifuge tube and then allow it to thaw while centrifuging.

The upper layer is slightly opalescent by transmitted light, and behaves like a suspension of anisotropic rods or plates that can be orientated by streaming. The stationary upper-layer fluid merely rotates the plane of polarization of polarized light, but fluid in which the particles have been orientated changes plane-polarized light into elliptically polarized light; solutions of myosin give a similar but weaker effect (Von Muralt and Edsall 1930). Those regions in the upper layer where there is a shear, and in which the particles are therefore orientated, become birefringent and are visible between crossed Nicol prisms; this is illustrated in fig. 2, Plate 12.

The lower layer is spontaneously birefringent (fig. 1, Plate 12); it may be perfectly clear by transmitted light, but it has a strong sheen by reflected light. The lower-layer fluid when separated from the upper can be mixed with a considerable volume of water and still be liquid crystalline if examined immediately in polarized light. A few minutes after diluting, however, it begins to change, and after standing for some time once more separates into two layers. The lower layer is again liquid crystalline, but now has a smaller solid content. Because of the small specific gravity differences involved the droplets of each layer move very slowly into the other, and it may take many hours for the interface between the two layers to become quite sharp (figs. 3 and 4, Plate 13). When the process of separation has started it can be accelerated by centrifuging, but the initial conversion of the unstable fluid into two layers proceeds faster if the

solution is allowed to remain quite still. The actual separation into layers also proceeds faster at 37° than at 0° C.

The process of diluting a bottom-layer fluid and separating off the new, more dilute bottom layer can be repeated until the concentration of solid in the bottom layer is reduced to about 1.6% with tobacco mosaic virus and Enation mosaic virus. With aucuba mosaic virus the limiting dilution is somewhat less, and the critical concentration is about 2.2%. These values probably have no absolute significance, and improvements in the methods of purification may lead to even lower figures. The critical concentration at which a solution becomes spontaneously birefringent is dependent to some extent on the temperature, and dilute solutions that are liquid crystalline at 0° C. may lose this property at room temperature and regain it when cooled.

In addition to being the more concentrated, bottom-layer material also seems to be the more pure, and the processes of dilution of bottom layer, which lead to a decrease in the concentration at which a virus solution will become spontaneously birefringent, usually also lead to an increase in the serological titre of the preparation. In general, however, there is no corresponding increase in the infectivity, suggesting that the units in the lower layer may possibly be larger aggregates than those in the upper, and there is no appreciable change in the analytical figures. Table II shows the results of three comparisons of the serological activity and the infectivity of top and bottom layers which separated from virus solutions prepared by precipitation with acid and ammonium sulphate.

TABLE II—ACTIVITY OF "TOP" AND "BOTTOM" LAYERS IN EQUILIBRIUM

Virus	Layer	Serological titre	Infectivity at various dilutions Average no. of lesions per half-leaf			
			10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
Tobacco mosaic	Top	1/6 × 10 ⁶	165	99	23	4
	Bottom	1/10 ⁷	161	80	25	2.6
Aucuba mosaic	Top	1/6 × 10 ⁶	144	51	24	5
	Bottom	1/10 ⁷	151	74	26	4
Enation mosaic	Top	1/3 × 10 ⁶	75	27	6.5	0.16
	Bottom	1/6 × 10 ⁶	72	27	7.5	0.5

Dilution given as weight of protein in grams per c.c.

Towards the end of the process of diluting successive bottom layers the difference in concentration between the two layers in equilibrium becomes smaller and smaller, and with tobacco mosaic and Enation mosaic viruses may be only one part in a hundred when the concentrations are about

1.8%. With more concentrated preparations the difference is much larger, and a lower layer containing 8% of solids may be in equilibrium with an upper layer containing only 4%. The precise values depend on the amount of impurities present.

From these dilution experiments it is apparent that solutions of tobacco mosaic and Enation mosaic virus purified by the use of strong salt solutions and acid can exist in the liquid crystalline state if they have a solid content greater than 1.6%, and that with aucuba mosaic virus a slightly higher concentration is necessary. If solutions of the purified viruses are not spontaneously birefringent when the solid content is from 2 to 3% they can be still further purified, if purity be estimated on the basis of serological activity. All virus preparations which give dilute liquid crystalline solutions, however, do not necessarily give uniformly high precipitation end-points. Some old preparations of tobacco mosaic virus with serological titres of only $1/4 \times 10^6$ became slightly gelatinous and gave spontaneously birefringent solutions with a solid content of but 1%, and the addition of certain substances, notably glycerophosphates and hexosephosphates, to fresh virus preparations has a similar effect. These phenomena, however, have not been investigated in detail.

The further purification of solutions of these viruses which, although relatively concentrated, are not liquid crystalline cannot be effected by precipitations with either acid or ammonium sulphate, for the contaminants (presumably breakdown products of the virus) precipitate under the same conditions and in the same apparently crystalline manner as the virus. For further purification it is necessary either to dilute successive fractions of bottom layer as described above, to incubate with trypsin, or to separate a product by centrifuging at high speed. The last method will now be discussed in more detail.

BEHAVIOUR OF THE VIRUS PREPARATIONS IN A CENTRIFUGAL FIELD

The general appearance of solutions of the three virus strains is the same whether they are dissolved in water or in dilute salt solutions, and the viruses cannot be removed from such neutral solutions by centrifuging at 3500 r.p.m. When either the salt concentration or the acidity is increased the fluids become opaque, and at a salt concentration that depends somewhat on the purity of the virus preparation and on the salt used it becomes possible to sediment the virus at this speed. Virus solutions to which insufficient acid for complete precipitation has been added have a different appearance from those to which insufficient salt has been added.

With acid the fluid becomes opaque and slightly rigid, but it shows no very noticeable sheen; this slightly gelatinous fluid is thixotropic. With a little ammonium sulphate the fluid becomes somewhat gelatinous and develops a very intense sheen, the sheen being obvious even when the fluid is at rest. Virus preparations sufficiently purified to give a birefringent layer at a concentration of 4% will give a precipitate which can be centrifuged out at 3500 r.p.m. when they are only one-eighth saturated with ammonium sulphate. By increasing the concentration of ammonium sulphate to one-sixth or one-fifth saturation the precipitate can be sedimented much more rapidly.

The optimal precipitation point with acid was found by centrifuging virus solutions at various pH values, and testing the supernatant fluids for their infectivity and serological activity. In these tests no indication was obtained that virus activity could be separated from the protein, and the infectivity and serological activity of the supernatant fluids were found to be inversely proportional to the amount of precipitate obtained. The optimal precipitation point was found to depend on the salt content of the virus solutions. In the presence of a little salt, tobacco mosaic virus precipitates completely at pH 3.4, but it can be precipitated over a considerable range on either side of this point by intense centrifuging. This is in good agreement with the pH value given by Stanley (1936a) for the isoelectric point of tobacco mosaic virus, and with the results of Best (1936) and Eriksson-Quensel and Svedberg (1936). In the absence of salts, or with a salt concentration of less than M/50, however, the apparent isoelectric point moves to about pH 4.2, but becomes even less precise than it is in the presence of salt. As previously stated the salt-free precipitate at pH 4.2 differs in appearance from that obtained in the presence of salt at pH 3.4, and it is apparently amorphous. No differences large enough to be of value for distinguishing between the three virus strains have been detected.

Bechold and Schlesinger (1933) showed that it is possible to sediment tobacco mosaic virus from clarified infective sap and, assuming a specific gravity of 1.12, they calculated the diameter of the particle as 500 Å. Using Stanley's purified virus preparations Eriksson-Quensel and Svedberg (1936) have found a similar value. Wyckoff and Corey (1936) have confirmed the fact that it is possible to centrifuge the virus out of clarified sap, and they have pointed out that the precipitate is birefringent. We have described briefly (Bawden, Pirie, Bernal and Fankuchen 1936) the separation of a jelly-like layer by centrifuging our purified virus solutions. In the experiments to be described below an Ecco-Blitz angle centrifuge, giving

a centrifugal field of 16,000 times gravity at the bottom of the tubes turning at a speed of 14,000 r.p.m., was used; it is therefore difficult to calculate values for the sedimentation constant from the data obtained. Furthermore, the viscosity and degree of mixing of the fluids centrifuged are uncontrolled factors. The optical properties of purified virus solutions suggest that the particles are rod-shaped, and it would, therefore, be impossible, at the present time, to deduce the particle size from the sedimentation constant even if the latter were measured accurately. Valuable centrifugal data can perhaps be obtained from untreated clarified infective sap if it should prove that the virus particles occur there in a more or less spherical state, but it has not seemed worth while to give any great precision to measurements on the purified and probably aggregated material, nor to look for minor differences in the sedimentation rates of the three virus strains.

When clarified infective sap is centrifuged at this speed a birefringent jelly-like layer is deposited. The rate of sedimentation, however, is slow, and as the volume of sap that can be centrifuged in this manner is small it is not at the present time a practicable method for the isolation of the virus.

Virus solutions in the two states which we have called top layer and bottom layer respectively behave differently when centrifuged at 14,000 r.p.m. Top-layer solutions deposit simply a viscous layer of jelly, the solid content of which depends on the concentration of the solution put into the centrifuge tube. By centrifuging for from 1 to 3 hr. it is possible to sediment most of the virus from a neutral solution, and to leave a more dilute solution of virus covered by a layer of water from 0.5 to 2 cm. deep. Table III shows the positive correlation between the solid content of the jelly and that of the fluid from which it was sedimented. Within the limits mentioned, the time of centrifuging does not affect appreciably the concentration of the jelly layer but only the total amount deposited. This is shown in Table IV. There is, of course, a small secondary effect produced by changes in the concentration of the fluid during the period of centrifugation.

Evidence that the particles in these purified virus preparations are built up by the linear aggregation of smaller particles will be presented later. Attempts to get centrifugal evidence for any variability in the length of the particles have so far failed. If the supernatant fluids from one centrifugation are poured off and then centrifuged again, they behave in exactly the same manner as did the original fluid at the same concentration. There is, therefore, no gross inhomogeneity in our preparations, but this method of testing is not sufficiently sensitive to detect an inhomogeneity of the

type reported by Eriksson-Quensel and Svedberg (1936). Similarly, if the supernatant fluid be divided into two, and one-half precipitated with ammonium sulphate and acid and then redissolved in its original volume of fluid, no differences can be detected between the centrifugal behaviour of the reprecipitated and the original solution by this method.

TABLE III—EFFECT OF THE CONCENTRATION OF THE CENTRIFUGED FLUID ON THE SOLID CONTENT OF THE JELLY DEPOSITED

Initial concentration of centrifuged fluid	Wet weight of sediment mg.	Dry weight of sediment mg.	% solid in sediment	% of total virus sedimented
A—Tobacco mosaic virus				
6.2	191	45	23.5	25
3.7	171	35	20.5	32
2.25	161	27	17	40
1.35	140	21	15	52
1.02	118	15	13	49
0.71	107	12	11	56
B—Enation mosaic virus				
4.0	128	36	28	30
2.6	119	31	26	40
1.0	77	17	22	57
0.7	52	10	19	48
0.53	41	7	17	44
0.41	35	5	14	41

Each tube contained 3 c.c. of fluid at pH 7, and was centrifuged for 2 hr. at 14,000 r.p.m. and at 23.3° C. Although concentrated, none of these fluids was spontaneously birefringent, i.e. they were all in the state we have called top layer.

TABLE IV—EFFECT OF DURATION OF CENTRIFUGATION AT 14,000 R.P.M. ON THE SEDIMENT

Time min.	Wet weight of sediment mg.	Dry weight of sediment mg.	% solid in sediment	% of total virus sedimented
A—2.5 % solution of tobacco mosaic virus				
192	160	40	25	53
127	96.5	24	25	32
58	39	10	25	13
23	12	3	25	4
B—1.3 % solution of Enation mosaic virus				
180	122	31.4	26	80
130	100	25	25	64
100	81	19	23	49
50	35	9	26	23

Each tube contained 3 c.c. of fluid, in the top-layer condition, at pH 7.

Table V shows the effect of centrifuging virus solutions of the same concentration at a number of different *pH* values. Below *pH* 5.6 the solutions become gelatinous, this being the first stage in the formation of the acid precipitate previously described.

TABLE V—EFFECT OF *pH* ON THE AMOUNT OF SEDIMENT

Buffer	Wet weight of sediment mg.	Dry weight of sediment mg.	% solid in sediment	% of total virus sedimented
<i>pH</i> 5.6 phthalate	179	52	29	75
<i>pH</i> 6.1 phthalate	128	37	29	54
<i>pH</i> 6.6 phosphate	115	33	29	48
<i>pH</i> 7.1 phosphate	104	32	31	46
<i>pH</i> 7.6 phosphate	96	30	31	43
<i>pH</i> 9.1 borate	93	28	30	41

Each tube contained 3 c.c. of top-layer tobacco mosaic virus 2.3 % and was centrifuged for 2 hr. at 14,000 r.p.m. in M/15 buffer at $23 \pm 3^\circ \text{C}$.

The spontaneously birefringent virus solutions which we have referred to as bottom layer centrifuge in a rather different manner. The jelly deposited may have less than half the solid content of that sedimented from a top-layer solution of the same concentration. There is no clearly defined interface between the jelly and the concentrated bottom layer that lies immediately above it; there is likewise no sharp interface between the bottom layer and the top layer which forms during centrifuging, but merely a diffuse region in which particles of the one layer are found dispersed in the other. A division, however, can be distinguished where the system changes from a suspension of top-layer material in bottom-layer material to a suspension of bottom-layer material in top-layer material.

The relationship that has been shown between the solid content of the jelly and that of the centrifuged fluid is interesting, for by varying the concentration of the centrifuged fluid it is possible to get stable jellies with very similar physical appearances, but with solid contents varying between 10 and 35 %. A rather limp jelly with a solid content of only 7 % has sometimes been precipitated. These jellies are highly birefringent, and are completely orientated when they are sucked into capillary tubes of about 1 mm. diameter. The results of X-ray examinations of these jellies and the nature of the forces which hold the particles apart as an oriented jelly in spite of the intense gravitational fields will be discussed later.

High-speed centrifugation can be used as a method of further fractionating these virus preparations. Preparations that have been partially inactivated by drying cannot be fractionated by this treatment, but those made by the

precipitation methods described, which will not give a birefringent layer unless more concentrated than 5%, will always deposit on high-speed centrifugation a jelly which dissolves to give birefringent solutions at about 2%. By this method of fractionation more than a half of the total virus can readily be recovered in a state in which it gives dilute liquid crystalline solutions, whereas if the further purification is carried out by the method of repeatedly separating and diluting successive bottom layers only a small part of the virus is recoverable in the final bottom layer. When, however, the risk of a possible contamination with trypsin is of no importance, incubation with trypsin is by far the most convenient method of obtaining the virus in the highly birefringent state.

Dilute solutions of the jellies obtained by high-speed centrifugation of top-layer material, on standing, again separate into two layers. Again, the lower layer is spontaneously birefringent while the upper is not. The serological activity and infectivity of top- and bottom-layer material obtained in this manner have been found to be the same. This fact is readily explicable if the jelly sedimented from partially purified preparations is homogeneous, i.e. if the high-speed centrifugation effects a further fractionation similar to that produced by dilution of successive bottom layers. The results of two tests on such layers are given in Table VI.

TABLE VI—COMPARISON OF THE SEROLOGICAL TITRE AND INFECTIVITY OF TOP AND BOTTOM LAYERS FROM DILUTE SOLUTIONS OF THE JELLIES OBTAINED BY CENTRIFUGING TOP-LAYER MATERIAL AT 14,000 R.P.M.

Virus	Layer	Serological titre	Infectivity on <i>Nicotiana glutinosa</i> Average no. of lesions per half-leaf			
			10^{-4}	10^{-5}	10^{-6}	10^{-7}
Tobacco mosaic	Top	$1/8 \times 10^6$	134	66	25	2.2
	Bottom	$1/8 \times 10^6$	129	72	23	2.6
Enation mosaic	Top	$1/6 \times 10^6$	122	45	8	2
	Bottom	$1/6 \times 10^6$	114	46	7	3

ABSORPTION SPECTRA

The ultra-violet absorption spectra of the three virus strains have been measured using a Spekker ultra-violet spectrometer and 2 cm. column of 0.02% virus solution. No definite differences have been observed between the spectra given by the different strains, and the spectra do not differ appreciably from those usually obtained with proteins. Fig. 5 is the absorption curve of a solution of aucuba mosaic virus.

SPECIFIC GRAVITY

The specific gravity of tobacco mosaic virus has been measured in three different ways. First, particles of the dried virus have been suspended in mixtures of nitrobenzene and dichlorobenzene, and the ratio of the two adjusted until the particles were in equilibrium. Secondly, the precipitate obtained by adding ammonium sulphate to virus solutions has been equilibrated in mixtures of sucrose and ammonium sulphate solution (Adair and Adair 1936). Thirdly, the specific volume of the virus has been

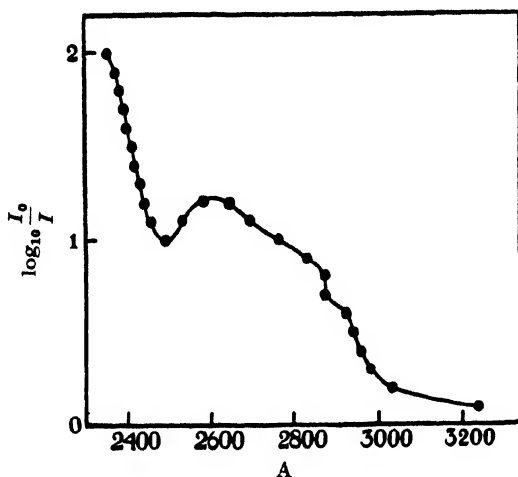


FIG. 5

determined from the specific gravity of a dilute solution of known concentration. The first two methods gave the specific gravity as 1.29–1.31, and this is in good agreement with the figure usually obtained with proteins.

Eriksson-Quensel and Svedberg (1936) have stated that the specific volume of tobacco mosaic virus protein is 0.646, i.e. the specific gravity is 1.55, and they comment on the fact that this is an unusual value for a protein. Our chemical observations on the virus preparations have shown no abnormality that might account for such a high specific gravity, and our measurements at 20° C. have given a specific volume of 0.73. This figure corresponds to a specific gravity of 1.37, and agrees well with the values obtained by this method with other proteins (Chick and Martin 1913). The differences that we have found between the specific gravity values as determined by the different methods also agree well with their observations. All three virus strains have given the same values for specific gravity.

Table VII shows the results of a few determinations of the specific volume of the three strains. These were measured by adding water to a 3.4 % solution of neutral salt-free virus until a glass float was in equilibrium at the temperature stated. The specific gravity of the float at that temperature was then measured by equilibrating it in solutions of sodium chloride of known concentration, and the values given in Landolt and Börnstein's tables for the specific gravity of sodium chloride solutions were used. From 1 to 2 c.c. of the virus solution was then frozen, dried and weighed to obtain its exact concentration. The value given in Table VII for a 26 % solution of tobacco mosaic virus was derived from a direct observation of the weight of a known volume of virus jelly.

TABLE VII—THE SPECIFIC VOLUMES OF THE VIRUSES

Strain	Concentration in g./100 ml. of solution	State	Temp. ° C.	Specific gravity of virus solution	Specific volume of virus
Tobacco mosaic	1.73	Top	20	1.0029	0.73
	1.88	Top	20	1.0033	0.73
	26.0	Jelly	15	1.07	0.73
Enation mosaic	1.61	Top	18	1.0025	0.76
	1.75	Top	19	1.0032	0.73
	2.49	Top	12.5	1.0065	0.72
	2.85	Bottom	18.5	1.0063	0.73
Aucuba mosaic	1.61	Top	16	1.0030	0.75
	2.78	Top	15	1.0064	0.74
	2.95	Bottom	20	1.0062	0.73

GELS, FIBRES AND "CRYSTALS" OF THE VIRUS

The satin-like sheen produced in solutions of these purified viruses when they are acidified or mixed with a quarter of a volume of saturated ammonium sulphate solution suggests that the material precipitated may be in the crystalline state. The individual particles of the precipitate, however, are too small to permit a conclusive microscopical examination. Attempts have been made, therefore, to increase the size of the "crystals" by slow precipitations; these attempts failed, but in the course of the work it became increasingly obvious that the first process in the production of the "crystals" is the formation of a gel, and that this breaks up when stirred or shaken and in its place are formed the small "crystals" or fibres with the characteristic sheen.

Phthalate buffer at pH 3.3 has been diffused into neutral virus solutions,

acetate buffer at *pH* 4 has been diffused into acid virus solutions and half-saturated ammonium sulphate solution has been diffused into neutral virus solutions. In each experiment a skin of jelly first precipitated at the interface, and then the remainder of the virus solution gradually turned to a limp jelly with no crystalline appearance until it was shaken or stirred.

When one volume of saturated ammonium sulphate solution is added to six or seven volumes of a dilute virus solution no precipitate is produced, but the fluid develops a very intense sheen and becomes slightly thixotropic. If this fluid is now allowed to evaporate slowly over calcium chloride it does not alter appreciably in appearance, but it is soon converted into a rigid jelly. This jelly when broken up by stirring changes into the usual "crystalline" precipitate got with ammonium sulphate.

The process of gel formation can be more easily studied in mixtures containing 0.5–1.0% of the virus, 0.25–0.5% of glycine and 5% of neutralized ethyl formate. The *pH* is adjusted initially to about 5, and the fluid is immediately put into the vessels in which it is to be observed. After a time, depending on the initial *pH* value, the glycine concentration and the temperature, sufficient ethyl formate will have undergone hydrolysis to give a mixture at about *pH* 3.3. During the acidification the optical properties of dilute virus solutions which have been kept quite still undergo no change, although the mixture turns to a fairly rigid jelly. When shaken or stirred this jelly breaks up and changes almost instantly to a suspension with the usual sheen. If now allowed to remain quite still the mixture does not return to the gel state, but it behaves exactly like the precipitate that is produced by adding acid directly to virus solutions, and it has the same appearance under the microscope. When more concentrated virus solutions are slowly acidified in this way they behave somewhat differently, for the undisturbed gel breaks up into a mosaic of irregularly disposed birefringent spindles. When stirred, however, this again gives rise to a suspension with the characteristic sheen.

These phenomena suggest that it is more accurate to describe the visible virus precipitates that are obtained with acid and ammonium sulphate as fibrous rather than as crystalline. The regular appearance of the fibres under the microscope could then be attributed to the more or less regular breaking up of a gel rather than to the wholly regular process of crystal formation (cf. Bernal and Fankuchen 1937).

From most points of view this distinction is not perhaps an important one, but if it be accepted it prevents us from adducing the apparent crystallinity of these virus preparations as evidence for their purity. Certain treatments can destroy infectivity without greatly affecting the physical

properties of these viruses. Such preparations give both ammonium sulphate precipitates and liquid crystalline solutions similar in appearance to those given by fully active virus. Other treatments, such as heating or exposure to alkali, which inactivate and dephosphorylate the protein, lead to a loss of the ability to give these crystalline appearances. Preparations partially inactivated by these methods, and therefore having a rather low activity and phosphorus content, may give with ammonium sulphate an appearance of crystallization very similar to that seen in fully active virus preparations. These contaminated preparations cannot be purified by further precipitations (or "recrystallizations") with ammonium sulphate, but they can be further purified by the other methods we have described.

THE EFFECT OF HEAT ON THE VIRUS PREPARATIONS

The coagulation of crude infective sap when heated to 70° C. has already been described. When dilute solutions of the purified virus preparations, protected from evaporation, are heated at 70° C. for as long as 30 min. there is no perceptible change in their appearance or in their ability to give anisotropy of flow. When tested at 60–70° C. dilute solutions give rather less anisotropy of flow than when examined at room temperature, but when cooled they are found to be unchanged. Similarly, if more concentrated solutions consisting of mixtures of top and bottom layer are heated to 70° C. the ratio of the two layers is unaltered.

When neutral solutions are heated to higher temperatures (75–80° C.) a fairly rapid change sets in; the fluid becomes opaque and soon turns to a gel, the rigidity of which naturally depends on the concentration of the virus solution heated. Two minutes at 80° C. will turn a 2% spontaneously birefringent solution to one showing only anisotropy of flow, and 4 min. will carry the decomposition far enough to render the fluid opaque. No differences have been noticed between the thermal labilities of the three virus strains.

Heating for a few minutes at 90–95° C. causes a coagulation and a complete loss of the ability to give anisotropy of flow: this treatment also destroys the infectivity and serological activity of the virus preparations. When a neutral solution in the presence of a trace of salt is heated for 5 min. at 95° C. the pH shifts slightly to the acid side, and the major part of the protein precipitates as a coagulum that can be centrifuged off. This precipitate is free from both phosphorus and carbohydrate. The supernatant fluid still contains some protein, part of which can be precipitated by the addition of from 0.5 to 1.0% of a neutral salt such as sodium chloride or

ammonium sulphate. The supernatant fluid now contains very little protein but all the phosphorus and carbohydrate content of the virus preparations. When mineral acid is added to the supernatant a curdy precipitate separates immediately, and soon aggregates into resinous masses with the characteristic appearance of nucleic acid. When denatured by heating (or by the other agents such as glacial acetic acid and pyridine, the effects of which will be described later) protein and nucleic acid are the only breakdown products that have been found. If there are any other constituents of these virus preparations they must be relatively minor ones. The properties of these two substances will now be described only superficially, for their more detailed examination must await the preparation of much larger quantities of the virus.

The proportions in which the soluble and insoluble proteins are found after denaturation by heating vary somewhat with the conditions of heating and with the *pH* value of the solution. The soluble protein can be removed by precipitation at *pH* 4.5, but if removed in this manner most of the nucleic acid is lost. When hydrolysed by acid these proteins behave like most other proteins, and the amino-nitrogen content of the hydrolysate reaches a maximum after 20 hr. boiling with 4*N* HCl. They give all the protein colour reactions, and have an arginine content (as estimated by Weber's (1930) modification of the Sakaguchi reaction) of from 6 to 7%. This reaction was used to follow the distribution of the protein in most of the experiments on the precipitation or enzymic hydrolysis of the protein. Negligible amounts of material were extracted from these proteins by prolonged extraction in a Soxhlet with ether, acetone, alcohol or pyridine. The proteins obtained from heat-denatured preparations of the different virus strains have shown no appreciable differences from each other.

The resinous masses of nucleic acid are readily converted to a loose powder by grinding with water. Neutral solutions of the sodium salt are convenient to handle, and they give a solid with a light open texture if dried while frozen. This material does not react with the virus antisera, nor does it in any way affect the precipitation of virus and antiserum.

Prepared in this way the nucleic acid product contains from 4 to 7% of phosphorus and from 24 to 30% of carbohydrate as estimated by the orcin method. Without previous hydrolysis it reduces potassium ferri-cyanide, under the conditions of the Hagedorn-Jensen reducing sugar estimation, to an extent corresponding to a reducing sugar content of 5%. It also gives biuret and Sakaguchi reactions corresponding to from 10 to 20% contamination with virus protein. This protein, judging from the Sakaguchi reaction, can be removed almost completely by prolonged

centrifugation at pH 3, or, less conveniently, by careful treatment with dialysed iron.

The purified virus nucleic acid resembles yeast nucleic acid closely; it contains a pentose and does not give the reactions with Schiff's reagent characteristic of a desoxy pentose. The phosphorus is liberated as phosphate on acid hydrolysis in two stages in the manner described by Jones (1920) for yeast nucleic acid. The question of the relationship between these virus nucleic acids and yeast nucleic acid will be dealt with in a later paper, but it may be said now that the molecule is larger than that of yeast nucleic acid prepared in the usual ways, for it is retained on collodion membranes which readily permit the passage of yeast nucleic acid. It is possible that this difference is simply the result of the more extensive degradation suffered by yeast nucleic acids during the course of isolation, for the methods used for the isolation of virus nucleic acid are much gentler than those necessary for the isolation of yeast nucleic acid.

EFFECT OF DRYING

It is well known that leaves of plants infected with strains of tobacco mosaic virus can be dried and still be infectious. Our preparations of the three strains are also active after drying in air or over phosphorus pentoxide. The activity, however, whether measured by the serological or by the infection method, is reduced to one-half or one-third by one such drying; by redissolving and then drying again the activity may be still further reduced. The loss in activity following on one drying over P_2O_5 is shown in Table I. After seven dryings in one experiment with tobacco mosaic virus the activity fell to approximately 1/100 of that of the original undried material. The loss of activity is the same whether the solutions are dried at neutrality or at the acid precipitation point. It is immaterial whether the solutions are dried unfrozen, or after being frozen slowly, or after being frozen rapidly in liquid air. Material dried frozen is, however, in the most convenient state for further handling, and we always use this method in getting dry weights. Frozen material which has been dried *in vacuo* over P_2O_5 for a day at room temperature loses less than 0.3 % of its weight on subsequent drying at 100° C. Repeated freezing and thawing has no apparent effect on the activity of these viruses.

Drying over P_2O_5 destroys to a large extent the ability of the virus to give liquid crystalline solutions, and also greatly reduces the readiness with which solutions will show the phenomenon of anisotropy of flow. For example, a solution, which was liquid crystalline at 1.8 %, when dried once

over P_2O_5 gave a solid which dissolved to give a solution containing only a trace of liquid crystalline material at 5.4 %, and none at 4.0 %. Virus that has been twice dried over P_2O_5 has never been observed to give a liquid crystalline solution at any concentration.

Solutions of dried virus resemble impure preparations of virus in many ways, and the properties of some unsuccessful preparations which are not highly active or which fail to give liquid crystalline solutions suggest that they are contaminated by virus breakdown products similar to those produced by drying the virus. The virus inactivated by drying precipitates in a manner similar to the active virus, and differs in this respect from the denatured product got by heating. Protein cannot be precipitated from a neutral solution of dried virus by the addition of a little salt, nor can nucleic acid be precipitated by acid. That part of the preparation which was inactivated by drying can be hydrolysed by commercial trypsin preparations, though much more slowly than can virus which has been denatured by heating. After digestion the part of the virus not inactivated can be isolated by precipitation with acid and ammonium sulphate. Without previous incubation with enzymes it has proved impossible to obtain virus from dried preparations giving either a high serological titre or capable of giving dilute liquid crystalline solutions either by precipitation with acid and ammonium sulphate or by centrifuging at 14,000 r.p.m. At this speed the dried material sediments in a similar manner and at about the same rate as the undried virus, but the precipitates obtained dissolve to give solutions which show anisotropy of flow only, and which are not liquid crystalline until more concentrated than 4–5 %.

This reproducible partial inactivation on drying is of interest because of the X-ray measurements on films of dried and partially dried virus. The course of the inactivation has therefore been followed in some detail. Samples of the same batch of tobacco mosaic virus were dried at atmospheric pressure over $CaCl_2$, $MgSO_4$ and $MgSO_4 \cdot 7H_2O$, Na_2SO_4 and $Na_2SO_4 \cdot 10H_2O$, and saturated ammonium sulphate solution. When re-dissolved the first solution was found to have lost half of its activity, the second one-quarter and the last two were unaffected. At 15° C. the mixture of $MgSO_4$ and $MgSO_4 \cdot 7H_2O$ establishes a water-vapour pressure of 5 mm. and the Na_2SO_4 mixture a pressure of 9.2 mm. (figures from Landolt and Börnstein's Tables). Similarly, there is no loss of activity or crystallinity on drying, or even on repeated drying, over the Na_2SO_4 , $Na_2SO_4 \cdot 10H_2O$ mixture in the vacuum attained by a water-pump. After drying over sodium sulphate the virus retains 15–20 % of water, this can be removed by subsequent drying over phosphorus pentoxide. Drying in

two stages in this way causes much less inactivation, whether measured by the capacity to give "bottom layer" or serologically, than direct drying over phosphorus pentoxide.

This curious behaviour can perhaps be explained in terms of the triangular shape deduced for the virus particles from X-ray measurements (Bawden, Pirie, Bernal and Fankuchen 1936), which indicated that the particles could pack together in two ways. It is possible that there is less mechanical destruction of the virus when the packing, which must take place during evaporation, is done gently.

The various methods of inactivating the virus seem to give rise to recognizably different products. By heating or treatment with acetic acid or pyridine, we get a nucleic acid and an insoluble denatured protein; when dried, a soluble nucleoprotein and no free nucleic acid; and when inactivated with acids or alkalis we get metaproteins and free nucleic acid.

The solid precipitated from fully active virus preparations at pH 3.4 in the presence of a little salt contains about 26 % of dry matter. In the absence of salts, even after long centrifuging at 3500 r.p.m. at pH values around the precipitation point, the precipitate cannot be packed so tightly. If, however, this loose sludge of virus is now drained without evaporation by centrifuging on a porous plate, the solid content can be raised to about 35 %. A jelly which also may have a solid content as high as 35 % can be deposited from neutral solutions of virus by centrifuging at 14,000 r.p.m. These treatments in no way affect either the activity of the virus or its capacity to produce dilute liquid crystalline solutions, and these are the greatest concentrations of virus we have obtained by mechanical means, i.e. without evaporation.

[*Note added in proof* 19 June 1937.—The acid precipitate can be converted into a translucent buttery mass with a water content of only 50 % by centrifuging for $\frac{1}{2}$ hr. at 14,000 r.p.m. in the end of an L2 Chamberland filter candle. When soaked in water this mass regains its "crystalline" appearance.]

THE EFFECT OF ENZYMES

No enzyme preparation has yet been found that attacks these purified virus preparations at an appreciable rate, or that has any permanent effect on their infectivity, and we have found incubation with trypsin to be a most effective method of further purifying our products. We have incubated the virus preparations with commercial trypsin, "pancreatin", pepsin, papain and autolysed preparations of kidney at a number of pH

values around the optima for enzymic activity. All these enzymes were shown to be strongly proteolytically active by testing them against heat-denatured virus which they rapidly hydrolysed, but they had no appreciable enzymic effect on the preparations of active virus. The behaviour of these strains of tobacco mosaic virus is, therefore, very different from that of potato virus "X" (Bawden and Pirie 1936), and our results are substantially the same as those obtained by Stanley (1934*a* and *b*) with crude infective sap. In the presence of large amounts of trypsin the infectivity of the purified virus preparations is almost destroyed while the serological activity is unaffected. This loss of infectivity occurs immediately the virus and enzyme are mixed, and no further loss follows incubation. By precipitation with acid or dilute ammonium sulphate solution, however, the virus can be recovered with its full activity from such non-infective mixtures. The material recovered from such mixtures seems to be identical with virus that has not been incubated with trypsin, but, as we have previously stated, the possibility that it may be a virus-trypsin complex cannot be definitely excluded.

THE RECOVERY OF VIRUS FROM PRECIPITATES WITH THEIR ANTISERA

When purified virus preparations are mixed with their antisera at optimal combining proportions a flocculent precipitate is produced, and the mixture is almost non-infective. A slight recovery of the infectivity can be made merely by diluting these mixtures. Chester (1936) has shown that the infectivity of crude infective sap and antiserum can be much increased by incubating them with pepsin.

If our purified virus preparations consist of a mixture of active virus and inert protein, or of a protein and a contaminating nucleic acid, it is to be expected that the precipitation with specific antisera might perhaps alter the ratio of the components. We have found no evidence for this, and material recovered from precipitates with antiserum after digestion with pepsin is apparently the same as our normal virus preparations.

The precipitate obtained by incubating 32 mg. of purified tobacco mosaic virus with 3 c.c. of antiserum was centrifuged off, suspended in water and heated for 5 min. at 78° C. The precipitate did not appear to coagulate, and after this treatment trypsin had little or no effect on it. After incubation at 37° C. at about *pH* 3 with pepsin, however, the fluid developed an intense anisotropy of flow, and after a few hours' incubation, material with the characteristic appearance of the purified virus preparations could be centrifuged off. 15 mg. of this material were recovered; it contained 0.5 %

phosphorus and 2.5 % carbohydrate, gave a serological titre of $1/4 \times 10^6$ and was infective at a dilution of 10^{-8} .

In a similar experiment using 50 mg. of a purified Enation mosaic virus preparation a final yield of 37 mg. was isolated. This showed good anisotropy of flow, had a phosphorus content of 0.48 %, gave a serological titre of $1/2 \times 10^6$ and was also infective at a dilution of 10^{-8} .

THE EFFECT OF VARIOUS SUBSTANCES ON THE VIRUS PREPARATIONS

Stanley (1935*b*) has described the effect of 110 substances on the infectivity of partially purified tobacco mosaic virus, and he has reviewed the literature on the subject in some detail. It appears from this that the agents which inactivate the virus are, in general, strong acids and alkalis, oxidizing agents and protein precipitants. In this section the effects of a miscellaneous group of substances on the purified virus preparations will be described.

Solvents

We have already shown that the virus is precipitated from plant sap by the addition of an equal volume of alcohol. When, however, salt-free solutions of the purified virus at pH 7 are mixed with alcohol there is no precipitate until the alcohol concentration is greater than 80 %. Denaturation proceeds slowly at this alcohol concentration. Concentrated solutions containing 30 % of purified virus can be dissolved in 80 % alcohol as easily as in water. The presence of salt, however, alters the behaviour greatly, and from certain salt solutions the virus can often be precipitated completely with 40 % alcohol. Calcium ions are more effective in producing this precipitate than the others that we have tried. Acetone has an effect similar to that of alcohol, but it denatures the virus more readily.

When dilute acetic acid is added to solutions of the three virus strains the characteristic acid precipitate already described is produced. When, however, solutions of virus are added to four or five parts of glacial acetic acid an irreversible change occurs, and there separates a precipitate of nucleic acid, apparently identical with that obtained by heating the virus preparations to 90° C. The clear supernatant fluid deposits on evaporation a phosphorus-free protein as a perfectly transparent, isotropic, tough film. This wets with difficulty and is only slightly soluble in water even when neutralized. The solution obtained does not react with the virus antiserum.

In small quantities pyridine has no effect on the virus preparations, but when the concentration is raised to 25–30 % a rapid denaturation sets in.

Initially such mixtures show the usual faint opalescence of the purified virus solutions, and they give anisotropy of flow, but after a few minutes they go quite clear and lose their ability to give anisotropy of flow. The addition of a little ammonium sulphate solution to this clear fluid causes the precipitation of denatured protein that is phosphorus-free and is soluble in water only in the complete absence of salts. From the supernatant fluid, nucleic acid can be precipitated by the addition of acid.

The instability of these viruses in the presence of solvents makes it difficult to assess the value of some of the analytical figures which have been published. The analyses have often been carried out on acetone-treated preparations, and no mention is made of the activity after this treatment. Such analyses may approximate more closely to analyses on phosphorus-free denatured protein rather than to analyses on the virus.

Urea

It is well known that concentrated solutions of urea readily denature proteins (Hopkins 1930), and they have also been found to inactivate many bacteria and bacteriophages (Burnet 1933). Precise measurements on the effect of urea on solutions of these viruses have not been made, but it may be said that they are unexpectedly resistant. They retain the ability to give spontaneously birefringent solutions in the presence of saturated urea for some hours, and several hours' exposure is necessary before any considerable part of the protein is denatured. This denatured protein is still largely soluble in water.

Nitrous Acid

It has been shown (Bawden and Pirie 1936; Bawden, Pirie and Spooner 1936) that nitrous acid destroys the infectivity of potato virus "X" preparations without affecting their serological activity. Stanley (1936*b*) has described a similar effect on tobacco mosaic virus and we have confirmed this.

Treatment with 15% acetic acid and 7% sodium nitrite for half an hour at 0° C. reduces the infectivity without affecting the serological titre. More vigorous treatment causes complete loss of infectivity, but there may also be some denaturation with consequent loss of serological titre and phosphorus. Treatments which just inactivate without denaturation do not affect the serological activity, the phosphorus content, or the ability of the preparations to give spontaneously birefringent solutions. Tobacco mosaic virus preparations inactivated by nitrous acid appear to be completely

unaltered antigenically, for tobacco mosaic antiserum absorbed at the optimal combining proportions with non-infective nitrite-treated tobacco mosaic virus will no longer precipitate with fully active virus.

Papain and Clupein

It has already been stated that papain has no enzymic effect on the purified virus preparations, but there is an interesting reaction between a constituent of commercial papain preparations and the purified viruses. When crude papain is extracted with water and the clear extract dialysed it is found that about one-tenth of the original material is indiffusible, and that this fraction retains the enzymic activity. A solution of this indiffusible material precipitates any of these virus strains from dilute neutral solutions, and the precipitate obtained resembles closely that obtained with acid or ammonium sulphate. Examined microscopically with dark ground illumination it has the same "crystalline" or fibrous structure. When varying amounts of papain were added to a constant amount of virus in one experiment it was found that the supernatant fluid, after centrifuging, gave a precipitate with neither papain nor virus when the two were mixed originally in the ratio of 1 of papain to 4 of virus. A similar, but amorphous, precipitate separates when papain is mixed with solutions of yeast, thymus or virus nucleic acids. From these precipitates the active enzyme may be recovered by extraction with acid. With the papain-virus precipitate the extraction should be made at pH 3.3, and with the yeast and thymus nucleic acid precipitates at pH 1.5.*

The precipitation of papain with both nucleic acids and the virus suggested that the protamines, which are well known to precipitate nucleic acids, might also precipitate the virus. It was found that a neutral solution of clupein sulphate will precipitate any of these virus strains at neutrality, and the precipitate does not become soluble until the pH is raised to 8.5 or 9. The virus, however, can be recovered readily by extracting the clupein from the precipitate with phthalate buffer at pH 3.3. When recovered in this way the virus has the usual phosphorus content, infectivity, serological activity and ability to give a spontaneously birefringent layer. The supernatant fluid from a mixture of virus and clupein sulphate contains neither substance when their initial ratio in the mixture is 20 to 1. When examined microscopically the virus precipitate with clupein resembles closely that produced with papain.

* The activity per milligram of the papain that has been extracted from a nucleic acid precipitate is greater than that of the original material. It is possible, therefore, that this process may be useful for the purification of papain.

The precipitates of all three virus strains with either papain or clupein are soluble in dilute salt solution, but there is a definite difference in the amount of salt necessary for the resolution of the precipitates with the different strains. The precipitate with aucuba mosaic virus does not occur if the concentration of sodium chloride is approximately $M/20$, but with tobacco mosaic virus and Enation mosaic twice this concentration is necessary to inhibit the precipitation. The precise values depend on the concentration of the two reagents and on the temperature, but the distinction between aucuba mosaic virus and the other two strains is clearer here than with any other physical or chemical properties that we have examined.

For a number of reasons these precipitates are of interest. They supply us with other methods for the isolation of these viruses, and possibly of other viruses and bacteriophages as well. For this purpose the precipitates with papain would probably be the more useful, for protamines give precipitates with a number of normal proteins (Lissitzin and Alexandrowskaya 1933). The fact that these insoluble complexes occur suggests that in making extracts from infected leaves some part of the total virus may be lost in the leaf tissue and in the cell debris. Finally, the phenomenon offers a clue to the possible nature of cell inclusions in virus-infected plants and animals, for protamines and histones are known to be constituents of most types of cells.

Effect of X-rays

Gowen and Price (1936) have shown that the infectivity of material obtained by drying clarified sap from tobacco mosaic plants can be destroyed by exposure to X-rays. The amount of virus inactivated was proportional to the amount of irradiation. We have obtained similar results with our purified virus preparations. These have been irradiated both as solutions and as dry powder with the same results. In one experiment with dry material, a 1 mm. layer had its infectivity reduced to about one-hundredth by 3 hr. irradiation, 8 cm. from a copper anticathode of an X-ray tube run at 30 kV and 20 mA. After 7 hr. exposure it was no longer infective. The type of inactivation resembles that following treatment of the virus with nitrous acid, for even after infectivity of the preparations is completely destroyed they still retain their serological activity. Also, when liquid crystalline solutions of the virus are irradiated these are unaltered in appearance and still birefringent when non-infective.

THE STATE OF THE VIRUS IN THE PLANT

The X-ray measurements made on the purified virus preparations show that the individual particles have a constant cross-section area of not less than 20,100 sq. A. It is not known whether the particles also have a constant length, but the extreme character of the orientation phenomena and the X-ray data indicate a minimum length of at least ten times the width. We have no evidence that particles of this length occur in the infected plant. Indeed from the results of experiments comparing the optical properties, activity and filterability of crude infective sap with those of solutions of purified virus it is more probable that the virus is in a different condition in the clarified infective sap, and that in the isolated product it has become aggregated.

The amount of anisotropy of flow shown by clarified sap varies somewhat with different samples, and it seems to be rather more definite with sap from plants grown in the winter. Although the yields of virus isolated from infected plants show that the infective saps must contain from 1 to 2 g. of virus per litre, yet the anisotropy of flow shown by clarified infective sap is usually much less than that shown by a 0.1% aqueous solution of the isolated virus. It is also much less than that given by a 0.1% solution of isolated virus in either clarified healthy tobacco sap or clarified infective sap, and the difference, therefore, is clearly not caused by the presence of protective colloids in the sap.

The relative activities of two samples of crude infective sap (or of two similarly treated solutions of isolated virus) are found to be the same whether they are compared for their serological titres or for their ability to produce local lesions, i.e. if one has twice the precipitation end-point with antiserum it will also be approximately twice as infective. When, however, a sample of crude infective sap and a solution of isolated virus giving the same serological titre are compared by the infection method the crude sap is found to be several times more infective. The results of three experiments comparing the serological activity and the infectivity of crude infective sap and 0.1% aqueous solutions of purified tobacco mosaic virus are given in Table VIII.

The filtration end-points of the virus before and after purification are also widely different. Smith and Doncaster (1936), using crude infective sap, obtained infective filtrates through collodion membranes with an average pore size of 53 m μ . This suggests a particle of diameter 17 m μ , or 170 A, which agrees well with the X-ray measurements of the width of the particles in our purified preparations. The purified virus preparations give

very different results. In aqueous solution at about pH 7 purified virus will not filter through Seitz pads or through collodion membranes with a pore size as great as $450\text{ m}\mu$; the filtrates through such membranes were protein-free, serologically inactive and non-infective.

TABLE VIII—COMPARISONS OF THE SEROLOGICAL ACTIVITY AND INFECTIVITY OF CRUDE INFECTIVE SAP AND 0.1% SOLUTIONS OF PURIFIED TOBACCO MOSAIC VIRUS

Virus preparation	Serological titre	Infectivity on <i>Nicotiana glutinosa</i> at various dilutions Average no. of lesions per half-leaf				
		1/10	1/100	1/1000	1/10,000	1/100,000
Crude sap	1/4000	180	88	45	4	0.8
Purified	1/8000	88	26	13	2	0.6
Crude sap	1/4000	255	168	75	25	5
Purified	1/10,000	208	118	27	5.3	1
Crude sap	1/1000	247	99	34	8	0.8
Purified	1/5000	139	46	17	2.6	0.6

All these results seem to indicate that in the purified virus preparations the individual particles are larger than those in expressed infective sap. Aggregation of the particles end to end would increase the ease with which they are orientated by streaming and so increase the amount of anisotropy of flow shown by a given amount of virus. It is to be expected that such an aggregation would affect also the filterability and infectivity, but not the serological titre to anything like the same extent.

The whole process of purification that we have described is not necessary to produce this apparent aggregation. After one precipitation of the virus from crude sap, with either alcohol, acid or ammonium sulphate, the amount of anisotropy of flow is definitely increased and the filterability is altered. Filterability and the amount of anisotropy of flow are closely correlated, increase in the latter being always accompanied by a decrease in the former.

The aggregated virus cannot be returned to its original condition by putting it into expressed plant sap. When added to clarified healthy sap the purified virus preparations still show quite definite anisotropy of flow, will not filter and their infectivity is not increased. Attempts to render the isolated virus more filterable by dissolving it in clarified infective sap also failed. Indeed, from the results given in Table IX it can be seen that the presence of the purified aggregated virus prevented the normal virus, which had not been precipitated, from passing the filters. In this experiment two samples of tobacco mosaic virus were filtered through a

membrane of average pore size, 450 m μ , approximately nine times the size normally required to stop tobacco mosaic virus. Sample 1 consisted of 5 c.c. of clarified infective sap plus 5 c.c. of phosphate buffer at pH 7, and sample 2 of 5 c.c. of clarified infective sap plus 5 c.c. of buffer containing 20 mg. of isolated virus. These were tested both before and after filtration for their infectivity and their serological activity.

TABLE IX—EFFECT ON THE FILTERABILITY OF VIRUS IN CRUDE SAP
BY ADDING ISOLATED VIRUS

	Serological titre	Infectivity on <i>Nicotiana glutinosa</i> No. of local lesions at two dilutions	
		1/100	1/1000
Clarified infective sap:			
Before filtration	1/320	419	78
After filtration	1/320	390	71
Clarified infective sap plus isolated virus:			
Before filtration	1/25,600	872	426
After filtration	1/20	33	2

Description of experiment in text.

We thus have little evidence as to the shape of the virus particles as they exist in the plants. It seems probable, however, that they have a width of about 17 m μ , and, as infective sap shows some anisotropy of flow, that the smaller units in the plant are either rods or plates or that in the plant a certain amount of aggregation into rods must occur.

In the section on the behaviour of the virus in a centrifugal field it was shown that birefringent jellies could be sedimented from clarified infective sap. Wyckoff, Biscoe and Stanley (1937) centrifuged both clarified infective sap and purified virus preparations, and found that the purified virus had the same sedimentation constant as the heavy constituents of the infective sap. At first sight this may seem to contradict our hypothesis that the particles become aggregated during the processes of purification, for larger particles would be expected to sediment more rapidly. If the particles were spherical this would be so, but no theoretical treatment of rod-shaped particles has yet been made to indicate the magnitude of differences which might be expected in the sedimentation rates of rods of the same width but of different lengths. In other words, it is unknown if the length of rods at all affects the sedimentation rate. That the observed sedimentation rate of the virus is the same after purification as it is before does not mean that the particles are necessarily the same size or that linear aggregation has not occurred, but the fact might equally well be advanced in support

of the view that the sedimentation rate of rods is relatively independent of their length.

[*Note added in proof 19 June 1937.*—We have found recently that the amount of anisotropy of flow shown by clarified infective sap can be greatly increased by centrifuging for from 2 to 3 hours at 14,000 r.p.m., and then resuspending the sediment in the supernatant fluid. This fact suggests that aggregation occurs as a result of high-speed centrifugation. Centrifugal data on untreated sap, therefore, is of questionable value as an indication of the size of the virus particle, unless it can be shown that the aggregation takes place only in the sediment and not during sedimentation.]

STUDY OF THE SAP FROM UNINFECTED TOBACCO PLANTS

We have been unable to isolate from uninfected tobacco or tomato plants any liquid crystalline proteins similar to those in our purified virus preparations. The protein content of clarified healthy tobacco sap may be only one-tenth to one-fifth of that of infective sap, and what protein there is precipitates differently. If the whole purification process described for the isolation of the viruses is carried out on healthy sap nothing is obtained.

The saps expressed from healthy and infected plants of the same age resemble one another closely both before and after clarification. When either acid or alcohol is added to the clarified saps both give precipitates. The precipitates, however, differ, for those from uninfected saps are darker in colour and only a small portion can be dissolved in water at neutrality. This soluble material is not precipitated by one-third saturation with ammonium sulphate, and the precipitate obtained with acid has not the characteristic satin-like sheen of the virus-containing precipitates. After long contact with ammonium sulphate the solutions often give a precipitate with a slight sheen, but this is merely calcium sulphate.

Both healthy and infective crude saps when heated to 70° C. give flocculent precipitates which aggregate rapidly and are easily removed by centrifuging. The supernatant fluids from healthy sap now contain very little material that can be precipitated with ammonium sulphate and contain only traces of indiffusible phosphorus, but the supernatant fluids from similarly heated infective saps contain the virus.

Healthy sap seems to vary from sample to sample more than infective sap, and we have sometimes found it impossible to isolate the nucleoprotein described below. When large amounts (up to two-thirds saturation) of ammonium sulphate are added to clarified healthy sap a precipitate con-

taining about 1 % of phosphorus often separates. This material (apparently a nucleoprotein, for it gives the protein colour reactions and contains phosphorus and carbohydrate) is quite soluble in half-saturated ammonium sulphate solution; it can be precipitated by acids and does not diffuse through cellophane membranes. Attempts have been made to exclude the possibility that this nucleoprotein may be a contaminant of our purified virus preparations and so responsible for their nucleic acid content. When the nucleoprotein from healthy plants is added to solutions of the isolated virus, the virus can be recovered alone quite easily by precipitation with dilute ammonium sulphate solution. The healthy plant nucleoprotein remains in the supernatant fluid, from which it can then be recovered quantitatively by precipitation with more concentrated ammonium sulphate solution. Unless, therefore, the two combine in a definite ratio it is improbable that our virus preparations are mixtures.

The nucleoprotein found in healthy tobacco plants is much less stable than the virus, and it has often disappeared entirely during the course of a few precipitations with acid or ammonium sulphate, and all the phosphorus has then been found in a form that is soluble in both acid and fully saturated ammonium sulphate solution. When heated to 90° C. the plant nucleoprotein, like the virus, breaks down, and gives a nucleic acid similar to that derived from heat-denatured virus. Proteolytic enzyme preparations that do not affect either the activity or the phosphorus content of the virus preparations readily destroy this normal plant nucleoprotein. This is further confirmatory evidence that our preparations are not mixtures of virus and a normal plant constituent, although it cannot be taken as proof.

We have, therefore, some evidence that this healthy plant nucleoprotein is not merely mixed with virus in our preparations, but no evidence that they are not combined. Our only evidence that the nucleic acid found in our preparations is an integral and essential part of the virus is the constancy of the observed phosphorus and sugar content of a large number of different virus preparations from a number of different host plants, and the fact that as yet we have been unable to get a fully active virus preparation that is phosphorus-free.

It is tempting to speculate on the possible part that this plant nucleoprotein may play in the multiplication of the virus, and to wonder if it is in any sense a precursor of the virus or whether it is one of the constituents of the healthy plant from which the virus is built up. There is no positive evidence for this belief, but the fact that a similar nucleoprotein has not yet been isolated from the sap of infected plants does perhaps favour it.

In addition to the differences in their protein content, the optical properties of saps from healthy and from tobacco mosaic plants also differ. When clarified infective sap is made to flow between crossed Nicol prisms it usually shows some anisotropy of flow, as was first recorded by Takahashi and Rawlins (1933*a*). We, however, have been unable to confirm their later statement that the sap from healthy tobacco plants shows a similar phenomenon (1933*b*). The amount of anisotropy of flow shown by infective sap can be much increased by precipitating the virus with alcohol or acid. The eluates from precipitates obtained by adding acid or alcohol to clarified healthy tobacco sap, however, show none.

DISCUSSION

Many of the implications of this work have already been discussed at some length. Of these, perhaps the most important are our justification for calling our isolated nucleoproteins "viruses" and the relationship between these products and the virus as it occurs in the plant. We may repeat that nucleoproteins with the characteristic optical properties which we have described have not been isolated from healthy plants, and no evidence has been obtained that virus activity can be dissociated from them. Also, the particular protein isolated is dependent entirely on the virus strain used and not at all on the host plant. This is strong presumptive evidence of the identity of our products, although not conclusive. It is, however, extremely difficult to account for the different behaviour of the virus before and after purification unless it behaves in the same manner as the isolated nucleoproteins. Stanley described the product he obtained by precipitation with ammonium sulphate as "a crystalline protein possessing the properties of tobacco mosaic virus". During the processes of purification, however, the virus undergoes a change that is not readily reversed, and loses completely the property that first distinguished tobacco mosaic virus as a new type of disease agent, namely, that it should pass fine filters. Also, the infectivity of the virus is considerably reduced. These phenomena can readily be interpreted if we assume that the virus particles have become aggregated linearly during the processes of purification. The behaviour of our nucleoproteins in solution, and the X-ray measurements, show that they are long particles such as would be produced by this type of aggregation. Further, their width is very similar to that found for the virus in crude sap by filtration experiments.

Since Stanley first described his "crystals", phrases such as "lifeless molecules" have been increasingly applied to viruses, and much has been

made of the idea that there is an essential incompatibility between the living and the crystalline states. As this is obviously an aesthetic rather than a scientific incompatibility it is necessary to be clear about the aesthetic connotations of the word crystalline. We have already suggested that the virus "crystals" might more accurately be described as fibres, and we doubt whether it is profitable to say that these viruses can be crystallized, or to apply the word crystalline to them without some qualification. Writers who find this incompatibility usually assume that a crystalline material must consist of a single definable chemical substance; this, however, is by no means necessarily true, for whenever groups of superficially similar substances are studied mixed crystals are found. The proteins form such a group, and, although it has been shown that the haemoglobins often do not form mixed crystals, the point has been so inadequately studied that it cannot be asserted that even true protein crystals are necessarily homogeneous. Structures such as plant fibres, hairs and muscle are fully as crystalline as the solid virus preparations have yet been shown to be, and the organization in suspensions of rod-shaped bacteria (or even shoals of fish) closely resembles that in the liquid crystalline virus preparations. A state of organization that is often described as crystalline is necessarily taken up by any collection of rods of equal cross-section when flowing or when packed tightly, and such states are widespread in nature (Schmidt 1924). Using crystallinity in this sense there is obviously no incompatibility between the living and the crystalline states, and it is only in the sense that any regularity in the arrangement of particles gives that arrangement some of the attributes of a crystalline substance that this term can be applied to the virus preparations. It is, however, unprofitable to attempt to apply the words living and dead to viruses, and here we are confronted with the wider question, which has been discussed elsewhere (Pirie 1937), of whether these words have any rigidly definable scientific meaning at all.

The X-ray measurements on soluble virus aggregates show that there is a regular arrangement of groups along each particle, and the sharpness of the reflexions shows that these particles are sufficiently long for a large number of repeat units to be found in each. Each particle has an internal regularity of the type sometimes found in large molecules, but with the viruses it is on an unusually large scale. In this sense the particle resembles an animal or plant fibre and may be spoken of as crystalline. When the soluble virus aggregates are aggregated still further by acid, ammonium sulphate or clupein, microscopically visible rod-shaped particles are formed. Suspensions of these visible rods show anisotropy of flow at even greater

dilutions than solutions of the purified virus. This suggests that the constituent rods of aggregated virus lie parallel in the "crystals", but there is no evidence that adjacent chains of particles bear any relationship to one another as they would in a true crystal. The arrangement in the visible virus aggregates may perhaps be compared with that of matches shaken to the side of a drawer, and the arrangement in a crystal with that of bricks in a wall. There is, as yet, no positive evidence in favour of either arrangement in the virus aggregates, and until evidence is obtained it is probably advisable to avoid the word crystalline in describing solid preparations of the tobacco mosaic viruses.* We see no reason why particles possessing many of the attributes of organisms should not pack into a three-dimensional lattice, and can see no essential incompatibility between crystalline states and those which could be described as living, but only a two-dimensional regularity has been demonstrated as yet in our virus preparations.

Virus preparations denatured by heat break down to give a nucleic acid and some denatured proteins, but no diffusible constituents have yet been observed. Because of this we described our products as nucleoproteins and, except for their capacity for linear aggregation, the description satisfies their chemical and physical properties. We have already stated that after denaturation by heat only protein and nucleic acid have been found. The apparent absence from these viruses of diffusible constituents separates them sharply from the bacteria. A further point of difference is the apparent absence of water from the interior of the virus particles even when they are in solution. It is not the fact that these viruses can be dried without losing all their activity that distinguishes them, for this is a property possessed by some organisms, but it is the fact that they normally occur with no internal water that is unusual. The X-ray measurements already published (Bawden and others 1936) show that the intramolecular spacings are the same whether the measurements are made on dilute virus solutions or on dried films of virus. Only the intermolecular spacings alter, and these are a function of the concentration of the virus solutions. From these results it follows that the internal composition of the individual particles is the same whether the particles are dissolved or in a dried film, for any picture that we may form of a structure able to expand sufficiently to contain an amount of water comparable with that usually found in bacteria, without in any way affecting the distance between the groups causing the X-ray reflexions, is too improbable to be taken at all seriously

* [Note added in proof 10 June 1937.—Bernal and Fankuchen (1937) now state definitely that the solid preparations of virus as yet studied are not true three-dimensional crystals.]

at present. The individual virus particles, therefore, seem to be solid masses of protein with no interstices, and this interpretation agrees well with the observed specific gravity of the material. In the matter of water content the particles much more nearly resemble protein molecules than they resemble either bacteria or the crystals of those proteins, such as the albumins, which have an open structure.

The fact that the individual particles contain no water makes it difficult to understand what forces hold apart the particles in the jellies which separate when the virus solutions are centrifuged at high speed, for, although they all have similar appearances, the jellies can have solid contents varying between 10 and 30 %. Regardless of the initial solid content, these jellies when diluted with water all give solutions that cease to be spontaneously birefringent at about the same concentration. From this fact we may argue that the constituent particles in all the jellies are of about the same length, by analogy with Staudinger's (1932) treatment of the behaviour of suspensions of rods at different concentrations. Staudinger has found that the graph relating the viscosity of a suspension of rods with its concentration has a sharp break at a concentration at which the rods have insufficient space for free rotation. It is reasonable to assume that in dilute solutions the anisotropic virus particles have sufficient room to move freely in all directions, and therefore are distributed at random. In bulk such dilute solutions will therefore be isotropic unless the random arrangement is interfered with by such forces as shearing. In virus solutions of a certain concentration, however, the virus particles are so close together that rotation about the two shorter axes becomes impossible, although translational motion is relatively unimpeded. At this concentration the fluid will consist of a three-dimensional mosaic of regions arranged at random to each other, but in each of which all the rod-shaped particles will lie approximately parallel. The concentration at which the isotropic virus solutions will become anisotropic (or top-layer fluids will become bottom-layer fluids) will depend on the length of the rod-shaped particles. It also depends on other factors, for impurities, such as virus breakdown products, can impede greatly the separation of the liquid crystalline layer.

When a layer of spontaneously birefringent virus solution is examined with a lens or a microscope through crossed Nicol prisms, the regions of parallel orientation are seen as coloured areas of different hues, for the colour given depends on the path difference between the ordinary and the extraordinary ray in each region, and this in turn depends on the thickness of the region and on the angle that the direction of the rods makes with

the plane of polarization. The size of the regions of parallel orientation varies inversely with the virus concentration. The reason for this is unknown, but with a little practice it can be used to estimate the concentration of bottom-layer virus solutions with some accuracy.

If Staudinger's theory can be applied to these virus preparations it is possible to obtain an estimate of the length of the rods into which the virus is aggregated during the processes of purification. At the critical concentration for the formation of a spontaneously birefringent virus solution the ratio of the volume occupied by the virus to that occupied by the water should equal the ratio of the volume of the rod to the volume in which the rod can rotate freely. The volume in which it can rotate freely cannot be less than that of a flat cylinder the diameter of which is the length of the rod and the depth of which is the width of the rod; and it cannot be greater than the volume of a sphere the diameter of which is the length of the rod. The X-ray measurements show that each rod has an effective diameter of 152 Å and an area of 20,100 sq. Å in the dry state, and we have already suggested that the area is unchanged by dissolving. For tobacco mosaic virus the critical concentration at which a solution becomes spontaneously birefringent is 1.6 %, and as the specific volume of the virus is 0.73, there are sufficient data to calculate the length of the rods. If the length of the rods be taken as $2L$, and if the volume required for the free rotation of the rod be a cylinder, then

$$\frac{100 - (1.6 \times 0.73)}{1.6 \times 0.73} = \frac{152 \times \pi L^2}{20,100 \times 2L},$$

while if the volume required for free rotation be a sphere, then

$$\frac{100 - (1.6 \times 0.73)}{1.6 \times 0.73} = \frac{\frac{4}{3}\pi L^3}{20,100 \times 2L}.$$

The two values of L that satisfy these equations are 7000 and 890, giving the length of the rods as 14,000 and 1780 Å. It is obvious that these figures can be used only to give the order of magnitude of the particle length, for a large number of assumptions have been made in the calculation. Staudinger argues that his solutions turn from the sol to the gel state when the volume available for each rod is much less than the volume of a flat cylinder. If we adopt this view, then the aggregated virus particles will have a length of 14,000 Å, and the reason for their not passing a membrane with pores of 4500 Å in diameter is readily understood.

All the layering and other phenomena have been studied in neutral aqueous solutions. As the isoelectric point of the viruses is considerably

on the acid side each rod will carry at pH 7 a large number of negative charges, for the apparent equivalent weight is only 4000, whereas a particle with a length of 14,000 Å and a cross-section of 20,100 sq. Å would weigh about 200,000,000 times as much as a hydrogen atom. The fact that the particles are so highly charged may account for the rigidity of the jellies which are sedimented when the virus solutions are centrifuged at high speed, and explain why the virus particles cannot be packed tightly by centrifuging. The large charge also seriously complicates a theoretical interpretation of the critical concentration at which the virus solutions become spontaneously birefringent.

The individual strains of tobacco mosaic virus cause quite characteristic symptoms in plants and it is obvious that they must differ from each other, but as yet we have noticed very few clearly defined distinctions. Of these, the difference in the solubility of the virus-clupein precipitates is the most definite. Bernal will report elsewhere that the three strains we have used give an X-ray pattern of identically spaced lines, but the intensities of the individual lines differ with the different strains. Wyckoff, Biscoe and Stanley (1937) state that aucuba mosaic virus has a sedimentation rate greater than tobacco mosaic virus and deduce from this that it has larger particles. This is difficult to correlate with our results. The X-ray measurements show that the two strains have the same area of cross-section and shape, and we have been unable to produce spontaneously birefringent solutions of aucuba mosaic virus as dilute as those of tobacco mosaic virus. If this property is a function of the length of the particles the observation suggests that the rods of aucuba mosaic virus are the shorter.

The physical and chemical properties and general appearance of solutions of the three strains are very similar. Stanley (1936*a*) in his paper on tobacco mosaic virus made no mention of a sheen in his preparations, but recently (1937) he has claimed that solutions of aucuba mosaic virus show a more pronounced sheen than solutions of tobacco mosaic virus, and that aucuba mosaic virus is more easily precipitated by ammonium sulphate and has an isoelectric point at pH 3.7 instead of pH 3.4. We are unable to agree with the first statement, for with a large number of different preparations of the three strains we have observed no consistent differences. The amount of sheen noticeable in a solution of given concentration, salt content and pH value varies with different preparations of the same strain, and becomes less if the solution is allowed to remain quite still for a long time or if it is centrifuged at high speed. Similarly, the ease with which the viruses can be sedimented in the presence of dilute ammonium sulphate solution varies greatly with different preparations of the same strain, unless the

preparation has been subjected to one of the treatments we have described for further purification. Differences noticed in the ease of precipitation with salts require cautious interpretation until the purity of the material used has been much more clearly defined.

The three different strains are closely related serologically, but antigenic differences can be found between them by cross-absorption experiments. These experiments will be described in a later paper.

The means by which these viruses increase in the plant are unknown. Stanley (1936a) suggests that the viruses are autocatalysts and cites trypsin and trypsinogen as an analogy. Although this view cannot be disproved there is some evidence against it. No protein has yet been found in healthy plants that is so similar to the virus as trypsinogen is to trypsin. Wyckoff, Biscoe and Stanley (1937) give the molecular weight of the virus protein, calculated from centrifugal data, as greater than 10,000,000, but they could find no particles in healthy sap with a molecular weight of more than 30,000. The amount of soluble protein in healthy tobacco plants is considerably less than that in infected plants. These facts make it improbable that the virus can catalyse the conversion of a normal soluble constituent of the plant into virus. Definite conclusions on the autocatalytic hypothesis can, however, only be drawn when we have much more information about the differences between those constituents of infected and healthy plants which are insoluble in water. It is possible that the virus is built up from molecules which are essential to the normal plant in small quantities; presumably if these were converted into virus the plant would be stimulated to produce more, and in this way the additional soluble protein in the infective saps could be explained. It is tempting to associate the materials from which the virus is reproduced with cell division or growth, for sap from actively growing plants is much richer in virus, and systemic symptoms are usually produced only in those parts of the plants that are growing. There is no positive evidence for this view, but the observation of Sheffield (1936) that mitosis precedes the production of local lesions in *Nicotiana glutinosa*, even when the leaves are mature and cell division has normally ceased, does perhaps support it.

Tobacco mosaic virus is one of the smallest and most stable of plant viruses. It differs widely from most others in many of its properties, and it is doubtful if the results we have described can be at all generally applied to plant viruses. Some presumptive evidence that the activity of potato virus "X" is connected with protein has already been described (Bawden and Pirie 1936), but that this protein differs widely from those derived from tobacco mosaic plants is quite evident from their different behaviour

in the presence of proteolytic enzymes. From cucumber plants infected with cucumber viruses 3 and 4 we have isolated nucleoproteins similar in many of their properties to those described in this paper (Bawden and Pirie 1937). The cucumber and tobacco mosaic viruses have different host ranges and differ in some of their physical properties. The cucumber viruses have at least one antigen in common with tobacco mosaic, and purified preparations readily show anisotropy of flow and give dilute liquid crystalline solutions. The ability of tobacco mosaic virus preparations to show these phenomena is much more closely linked with their serological activity than with infectivity, for preparations inactivated by heat, drying, acid or alkali lose their serological activity and their ability to give anisotropic solutions, but when infectivity is destroyed by some other agents, such as X-rays and nitrous acid, the preparations still retain their full serological activity and still readily show anisotropy of flow and form dilute liquid crystalline solutions.

We have great pleasure in thanking Mr. E. T. C. Spooner for preparing all the antisera used, Dr. K. M. Smith and Mr. J. P. Doncaster for doing the filtrations through collodion membranes, and Mr. J. D. Bernal for much help and advice.

SUMMARY

Nucleoproteins with characteristic optical properties have been isolated from solanaceous plants infected with three strains of tobacco mosaic virus but not from healthy plants. These proteins are infective at a dilution of $1/10^{10}$ and give specific precipitates with antisera at a dilution of $1/10^7$. Solutions of the highly purified proteins separate into two layers if the protein content is raised above about 2%. The lower layer is the more concentrated and is birefringent, while the upper layer shows anisotropy of flow. There is no essential difference in the virus activity, expressed in terms of solid content, of the two layers. The anisotropy of flow can be easily recognized in solutions containing only 0.02% of protein. When centrifuged at high speeds these solutions deposit the protein in the form of a birefringent jelly.

No enzyme preparation has yet been found which attacks these proteins at an appreciable rate, but the activity can be affected by a number of chemical agents.

The stability towards drying and heating has been studied and the conditions under which the nucleic acid/protein complex breaks down.

The physical properties of virus preparations and the X-ray measurements on them are interpreted on the theory that in purified preparations the constituent particles are rod-shaped, and it is suggested that these rods are built up by the linear aggregation of smaller units. There is evidence that, in the plant, part at least of the virus is not aggregated, for filters which pass an infectious filtrate with untreated plant sap do not do so with purified preparations.

The relationship between the nucleoproteins and the viruses is discussed.

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DESCRIPTION OF PLATES

Plate 12

- FIG. 1—Photograph, in polarized light, of a 2% solution of purified tobacco mosaic virus which has been allowed to settle. The lower layer is birefringent, whereas the upper shows only anisotropy of flow.
- FIG. 2—Two identical jets of 0.2% Enation virus solution are flowing into a glass-sided cell 6 mm. deep and full of the same virus solution. The cell has crossed polarizing screens on opposite sides, the plane of polarization of the incident light is horizontal. One jet, *A*, is flowing in the plane of polarization, whereas the other, *B*, is at an angle of 45° to it. The stream *B* shows clearly, whereas the stream *A* is not itself visible but only the turbulence on either side of the actual jet can be seen. From this it is clear that the appearance of a virus solution in polarized light does not give a true picture of the actual motion of the fluid. The actual motion can be deduced from it, however, if we know how the appearance of the individual anisotropic rods varies as they rotate about an axis lying in the plane of polarization. (Natural size.)

Plate 13

- FIGS. 3 and 4—Photomicrographs, in polarized light, of the interface between "top" and "bottom" layers in a cell 1 mm. deep. In fig. 3 the photograph was taken after 6 hr. and in fig. 4 after 24 hr. when the separation had become complete. The "bottom" layer is, as described in the text, highly coloured. (Magnification $\times 8$.)

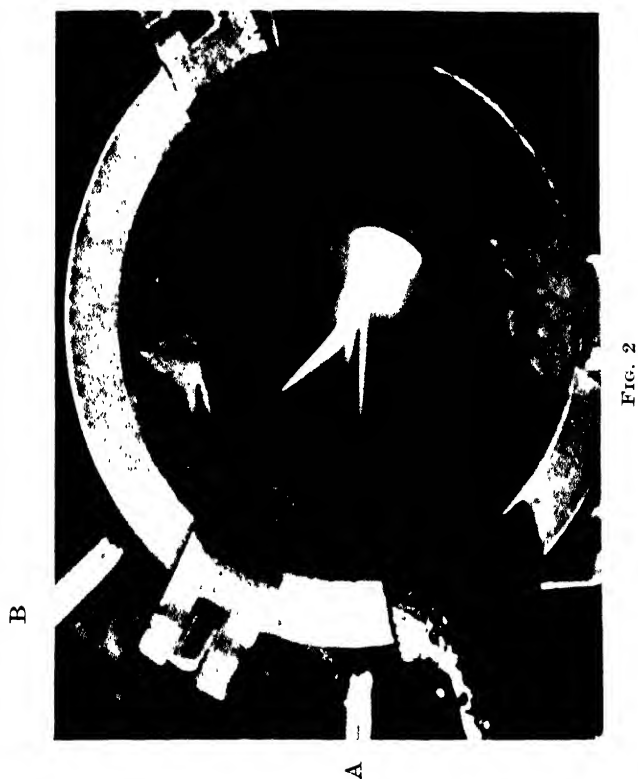




FIG. 3



FIG. 4

THE RELATIONSHIPS BETWEEN LIQUID CRYSTALLINE
PREPARATIONS OF CUCUMBER VIRUSES 3 AND 4
AND STRAINS OF TOBACCO MOSAIC VIRUS.

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FOUR viruses affecting cucumber plants have been described by Ainsworth (1935) as cucumber viruses 1, 2, 3 and 4. These fall into two pairs, the individuals of each pair possessing similar general properties. Viruses 1 and 2 are transmitted by aphids, are inactivated by 10 minutes' heating at 60° C., or by a few days' ageing in expressed sap, and are readily transmitted to members of the Solanaceæ and other families. Cucumber viruses 3 and 4 are apparently not transmitted by aphids, are not inactivated by 10 minutes' heating at 80° C., or by some months' ageing in expressed sap, and they have not been transmitted to any plants except members of the Cucurbitaceæ. A further fact which relates viruses 3 and 4 and separates them from 1 and 2 is that cucumber plants infected with virus 3 are resistant to further infection with virus 4, but are still quite susceptible to viruses 1 and 2. Viruses 3 and 4 are differentiated merely because they cause different symptoms. Cucumber plants infected with virus 3 show a general dark green mottle and the leaves

become somewhat blistered and deformed, while those infected with virus 4 show a bright yellow blotchy type of mottling and but little blistering or deformation.

Ainsworth's results suggest that the relationship between viruses 3 and 4 might be of the same type as that between the strains of tobacco mosaic virus, and they also show that the stability of the two viruses *in vitro* is of the same order as that of tobacco mosaic virus. As far as is known the recognized strains of tobacco mosaic virus and cucumber viruses 3 and 4 have no common host plants: we have been unable to infect cucumber plants with three different strains of tobacco mosaic virus; other workers have found the members of the Cucurbitaceæ to be immune to tobacco mosaic virus, and, as already stated, Ainsworth found the host range of cucumber viruses 3 and 4 to be restricted to the Cucurbitaceæ. Comparative infection experiments with the different viruses therefore cannot be made, and it is impossible to determine whether they are sufficiently related to immunize plants against one another. The results presented in this paper show that in spite of the wide differences in the host ranges of the two groups of viruses they are fairly closely related. They have antigens in common, and from cucumber plants infected with viruses 3 and 4 we have isolated nucleoproteins infective at high dilutions (Bawden and Pirie, 1937a). These nucleoproteins have similar analytical figures and many properties similar to those previously isolated from solanaceous plants infected with strains of tobacco mosaic virus (Bawden and Pirie, 1937b). The differences at present noted between cucumber viruses 3 and 4 and strains of tobacco mosaic virus, however, are considerably greater than those noted between the individual strains of tobacco mosaic virus.

PREPARATION.

The general methods described for the purification of the strains of tobacco mosaic virus have been used for the cucumber viruses, but as the latter proved rather more difficult to isolate, certain modifications of the original method have been made. The final yields obtained have also been less, averaging from 0.2 to 0.3 g. per l. of expressed cucumber sap as compared with about 2 g. with tobacco mosaic virus. Cucumber viruses 3 and 4 precipitate from plant sap at around pH 4.8, whereas the strains of tobacco mosaic virus precipitate at around pH 3.4. This fact possibly accounts for some of the difficulty experienced in freeing the cucumber viruses from plant proteins by precipitation methods. It is also of interest in view of the different host ranges of the two groups of viruses; for the pH value of expressed cucumber sap is between 7 and 8, whereas that of tobacco sap is between 5 and 6. Of the methods of preparation yet tried the following has proved most effective.

Cucumber plants are picked about a month after infection when they are showing most definite symptoms, minced in a meat mincer and the sap expressed through muslin. The mincing is easier and more sap is obtained if the leaves are first sprinkled with a dilute solution of sodium cyanide. This also has two other useful effects: it largely prevents the formation of highly coloured oxidation products which are difficult to remove from the final preparation, and it increases the yield of virus by raising the alkalinity of the extract. 0.5 to 1 g. of cyanide should be used for each kilogram of leaves. The expressed

sap is then heated to 70° C., and rapidly cooled. This treatment produces a flocculent precipitate, which aggregates quickly and is readily thrown down by centrifuging. The precipitate should be thoroughly washed in water and again centrifuged, for a certain amount of virus adheres to it.

The greenish-brown supernatant fluid is then brought to about pH 4·8 by the addition of HCl, and the precipitate produced is centrifuged off. The precipitate is suspended in water and dilute NaOH solution added to bring the pH to about 7·5, when the suspension is centrifuged. Sufficient ammonium sulphate is now added to the clarified supernatant to give a quarter saturated solution, and the precipitate formed is centrifuged off. This precipitate is taken up in water, centrifuged and the precipitate discarded; the supernatant fluid is then again one quarter saturated with ammonium sulphate. The precipitate obtained at this stage of the preparation is usually greyish in colour, and shows a pronounced satin-like sheen similar to that shown by preparations of tobacco mosaic virus. When examined microscopically (preferably with dark-ground illumination) it is seen to be composed of fairly regularly shaped needles, similar to those which Stanley (1936), working with tobacco mosaic virus, described as crystals. Bernal and Fankuchen (1937), however, have now pointed out that these needles lack the regularity characteristic of true crystals, and that they are ordered in two dimensions only. The precipitate is centrifuged down, dissolved in water and the solution again centrifuged. It easily comes clear by transmitted light, although retaining an intense sheen by reflected light. This fluid is diluted somewhat, and is then brought to about pH 4·8 by the addition of *N*/10 HCl, when a precipitate with the sheen is again produced. This is thrown down and then washed several times by stirring up with water and repeated centrifuging. When the water used for washing is nearly free from sulphate, the precipitate is dissolved by the addition of dilute NaOH solution. At this stage in the preparation of the strains of tobacco mosaic virus it was noticed that the precipitation point with acid showed a definite shift, and the precipitate obtained at pH 3·4 from dilute salt solutions became soluble at this pH when salt-free. This shift is presumably similar to the rather smaller shift in the same direction that has been observed with certain other proteins (Adair and Adair, 1934; Smith, 1936). Most preparations of cucumber viruses 3 and 4 have not shown a shift of this type, but a few were found to sediment more easily at about pH 5·5 when salt-free than they did at pH 4·8. In the complete absence of salt the viruses are not readily sedimented by centrifuging at 3500 r.p.m.

At pH 6 preparations of cucumber viruses 3 and 4 are definitely more turbid than are those of tobacco mosaic virus of the same concentration, but the turbidity disappears at above pH 7. Neutral aqueous solutions of the material prepared in this way, especially if young cucumber plants are used as a source of the virus, are sometimes quite colourless, and if more concentrated than about 3 p.c. separate on standing into two liquid layers. More often, however, preparations at this stage are quite definitely coloured and do not layer. Such preparations can only with difficulty be fractionated further by more precipitations with acid and ammonium sulphate. All the material in the solutions is precipitated by these treatments apparently in the paracrystalline form. Such preparations can easily be purified further either by

incubation with trypsin or by high-speed centrifugation. When preparations have been incubated with about 0.2 p.c. trypsin at 38° C. and at pH 8 overnight they have always given colourless neutral solutions which separated into two layers, after two or three further precipitations with acid or ammonium sulphate. Alternatively, the material can be fractionated by centrifuging the neutral solutions at 16,000 r.p.m. This treatment sediments a transparent jelly, which dissolves in water to give a colourless solution that separates into two layers.

The layering phenomenon in purified preparations of cucumber viruses is similar to that previously described with the strains of tobacco mosaic virus (Bawden and Pirie, 1937*b*). It has not been studied in such detail with cucumber viruses 3 and 4, but as yet it has been observed at room temperature only in solutions containing more than 2.5 p.c. of solids. At 0° C. layers can separate from more dilute solutions. The upper layer is the more dilute and by transmitted light is faintly opalescent; the lower layer may be quite clear by transmitted light but generally has an intense sheen by reflected light. When solutions are left undisturbed for some months the lower layer loses the sheen, but it regains it if it is shaken with the upper layer and then again allowed to layer; similarly, if a sample of the lower layer is centrifuged at 16,000 r.p.m. for half an hour a layer of perfectly clear liquid crystalline solution appears in the middle of the tube between the sedimented jelly at the bottom and the very dilute solution remaining at the top. This clear solution shows no sheen. It seems, therefore, that the sheen of the bottom layer is a result of incomplete separation, and is caused by droplets of top-layer fluid suspended in it, and is not a necessary consequence of its liquid crystallinity. The upper layer is not spontaneously birefringent, but readily shows the phenomenon of anisotropy of flow, *i. e.* it becomes birefringent when agitated or when flowing. The jellies which sediment when neutral or acid solutions of cucumber viruses 3 and 4 are centrifuged at high speed are also birefringent.

The sheen and the phenomenon of anisotropy of flow suggest that these particles, like those of tobacco mosaic virus, are rod-shaped, and this has been confirmed by the X-ray measurements of Bernal and Fankuchen (1937).

ANALYSIS.

Dried preparations of cucumber viruses 3 and 4 closely resemble similarly treated preparations of strains of tobacco mosaic virus. When dried in the frozen state the material has a light open texture and is easily handled. After drying the material is still infective, but its ability to show the phenomenon of anisotropy of flow is much reduced and its serological activity is reduced to about one-half. The analytical figures obtained have varied slightly from preparation to preparation, but normally fall in the following ranges:

Carbon	50.00 to 51.0 p.c.
Hydrogen	7.10 „ 7.6 „
Nitrogen	15.30 „ 15.8 „
Sulphur	0.00 „ 0.6 „
Phosphorus	0.55 „ 0.6 „
Carbohydrate	2.20 „ 2.5 „
Ash	1.00 „ 2.0 „

The phosphorus and carbohydrate contents were estimated by the methods previously described (Bawden and Pirie, 1937*b*). The phosphorus and carbohydrate can be isolated as nucleic acid after these viruses have been inactivated by heating, in the same manner as previously described for the strains of tobacco mosaic virus. The intact nucleic acid + protein complex resists attack by trypsin preparations containing nuclease, but when the complex has been disrupted by heat the components are readily attacked by this enzyme mixture. Schmidt (1936) has found other nucleoproteins to behave in this way.

Further evidence that the nucleic acid and protein are combined and not merely mixed in the virus preparations, as Stanley states (1937*a* and *b*), was obtained by analysing the precipitates obtained by centrifuging the virus preparations at a high speed. When solutions of the cucumber viruses are centrifuged at 16,000 r.p.m. at pH 8 and pH 3 birefringent jellies are deposited. After drying, both these have the same nucleic acid content as the material that is obtained by centrifuging at low speeds at the acid precipitation point. Stanley (1937*b*), in an attempt to show that the protein he had isolated was tobacco mosaic virus and not a mixture of protein and virus, centrifuged his products at a number of pH values on either side of the iso-electric point, and showed that the sedimentation rate of the virus (measured by infectivity tests) was the same as that of the protein. From this he argues that the virus is not merely a contaminant adsorbed on to the protein, for if it were it should come off when the pH is sufficiently altered. If this is a valid argument it should also have been possible to separate the nucleic acid from the protein in our experiments by altering the pH if the two components were merely mixed.

The only evidence against our conclusion that the isolated materials are nucleoproteins is in two of Stanley's statements. Firstly (1936), he stated that his preparations of tobacco mosaic virus contained no phosphorus, and secondly (1937*b*), that, as usually prepared, they contain phosphorus and carbohydrate in the form of nucleic acid, but that this is inessential for virus activity and can be removed by prolonged alkaline dialysis. Best (1936) has shown that the infectivity of tobacco mosaic virus preparations is reduced to one half by 12 hours' exposure at room temperature and pH 8.2. Stanley has published no figures to show that the serological activity and infectivity of his virus preparations are unaffected by the treatments used to remove the phosphorus and carbohydrate. In our experience those treatments which lower the phosphorus content to a level at which its detection becomes difficult, *i.e.* 0.05 p.c. or lower, invariably lead to a corresponding decrease in the activity of the virus preparations. The resulting products are still highly infective, *e.g.* infections may be obtained with as little as 10^{-8} or 10^{-9} , but it seems reasonable to assume that these infections are brought about by the remaining, undecomposed nucleoprotein, and not by the dephosphorylated protein which constitutes the bulk of such material.

ACTIVITY AND SEROLOGICAL REACTIONS.

No host plants are known in which cucumber viruses 3 and 4 produce local lesions, and the quantitative methods used for determining the infectivity

of the tobacco mosaic virus preparations at various dilutions could not be employed. The infectivity of the purified cucumber viruses was therefore tested merely by rubbing the leaves of cucumber plants with 1 c.c. of solutions containing various amounts of nucleoprotein, and noting the greatest dilution at which systemic infections were obtained. The results of six tests of this type are shown in Table I, and it will be seen that the smallest amount required to produce infection has varied from 10^{-8} to 10^{-10} g.

TABLE I.—*Infectivity of Purified Preparations of Cucumber Viruses 3 and 4.*

Virus.	Dilution.			
	10^{-7} .	10^{-8} .	10^{-9} .	10^{-10} .
Cucumber 3 . . .	++	++	++	--
„ 3 . . .	++	++	++	+—
„ 3 . . .	++	++	+—	--
„ 4 . . .	++	++	+—	+—
„ 4 . . .	++	++	++	--
„ 4 . . .	++	++	--	--

The dilution is given in grammes of protein per c.c. of inoculum and two plants were inoculated with 1 c.c. each at every dilution. The + sign indicates systemic infection and the — sign indicates that the plant remained healthy.

Tobacco (var. White Burley), tomato, *Nicotiana glutinosa*, and Golden Cluster beans, which are all susceptible to tobacco mosaic virus at high dilutions, did not become infected when inoculated with solutions containing as much as 1 p.c. of the nucleoproteins isolated from infected cucumber plants.

The purified preparations of cucumber viruses 3 and 4 are antigenic, and antiserum precipitating at a dilution of 1 in 1000 were produced by giving rabbits a single intravenous injection of 5 mg. With these antisera the serological titres of the virus preparations were determined by the technique previously described, 1 c.c. of virus solution at various dilutions being mixed with 1 c.c. of antiserum at a constant dilution. The highly purified, liquid crystalline, preparations of cucumber viruses 3 and 4 have given precipitation end points of from $\frac{1}{6 \times 10^6}$ to $\frac{1}{8 \times 10^6}$. The activity is, therefore, very similar to that of purified tobacco mosaic virus, and the serological titres are of the same order as those obtained with other highly purified antigens.

The serological titre of the material as normally prepared gives an accurate index of its infectivity, but certain treatments, *e.g.* with nitrous acid or exposure to X-rays, destroy infectivity without affecting the serological reactions. Preparations of cucumber viruses 3 and 4, rendered non-infective by such methods, still show anisotropy of flow and form liquid crystalline solutions, and when precipitated with acid or ammonium sulphate form paracrystals indistinguishable from those of fully active virus.

The infectivity of our purified virus preparations is not sufficiently great for us to state that they contain only virus particles, and that the nucleoproteins are therefore necessarily the viruses. The high serological titres and data from centrifuging and X-ray experiments suggest that our material is not grossly contaminated, but there remains the possibility that the nucleoproteins

we have isolated are antigens peculiar to the infected plants, and that they are "contaminated" with small amounts of virus responsible for the infections. If this is so, then we must assume that in addition to the viruses themselves multiplying in the infected plants they also produce (or cause to be produced) the nucleoproteins, for these have not been isolated from healthy plants. We have no evidence that infectivity can in any way be dissociated from the nucleoprotein: infectivity is closely correlated with the amount of protein, and loss of infectivity is accompanied by changes in the protein, and denaturation of the protein by heat, acid or alkali is accompanied by loss of infectivity.

At the present stage of the work it seems most reasonable to assume that the nucleoproteins are the viruses, but to remember that the point is not proved, and the possibility still exists that the nucleoproteins are disease products to which the viruses are firmly attached.

Cross-precipitation tests with cucumber virus 3 as antigen and tobacco mosaic virus antiserum, and *vice versa*, have shown that the two viruses are serologically related, for both antigens precipitate with both antisera. The serological titres given by preparations of either virus are practically independent of the antiserum used, but the range of antigen dilution over which precipitation takes place varies greatly with different antisera. When the cucumber viruses are titrated against a constant amount of the antiserum to any of the three strains of tobacco mosaic virus which we have used, there is a central zone of precipitation with large zones of non-precipitation in the antigen excess region (see Table II). When any of the three tobacco mosaic viruses are titrated against a constant amount of antiserum to cucumber virus 3 at the same dilution there are similar zones of non-precipitation in the antigen excess region. When cucumber viruses 3 and 4 are titrated against cucumber virus 3 antiserum, or when any of the three strains of tobacco mosaic virus are titrated against either their own or each other's antiserum, the zones of non-precipitation in the antigen excess region occur only where the antigen is very much more concentrated, and the zones are therefore smaller. The results of an experiment in which tobacco mosaic virus and cucumber virus 3 were titrated against the four antisera are shown in Table II. It will be seen that there are differences in the range of precipitation of the antisera to the individual strains of tobacco mosaic, but these are extremely small in comparison with the differences between the tobacco and the cucumber viruses.

Many virus workers have shown that the individual strains of plant viruses are serologically related, and at the present time it seems that all viruses which have the power of immunizing plants against one another are serologically related. Only Chester (1936) has shown that the strains are not serologically identical. Chester, using clarified infective sap as his antigens, found that he could absorb tobacco mosaic virus antiserum with *Aucuba* mosaic virus and then get no precipitate with the latter, but still get a precipitate with tobacco mosaic virus. Similarly, if *Aucuba* mosaic virus antiserum was fully absorbed with tobacco mosaic virus it would still precipitate with *Aucuba* mosaic virus. Chester also found that some of the strains isolated by Jensen (1933) were serologically identical with tobacco mosaic virus and others with *Aucuba*

TABLE II.—*Precipitation of Tobacco Mosaic Virus and Cucumber Virus 3 with Different Antisera.*1. *Tobacco Mosaic Virus as Antigen.*

Antiserum.	Time.	Dilution of antigen (1/1=1 mg. per c.c.).					
		1/1.	1/4.	1/16.	1/64.	1/256.	1/1024.
Tobacco mosaic virus	2 min.	++	++	+	—	—	—
	2 hr.	++	++	++	++	++	+
	24 "	++	++	++	++	++	++
Aucuba mosaic virus	2 min.	++	++	+	—	—	—
	2 hr.	++	++	++	++	++	+
	24 "	++	++	++	++	++	++
Enation mosaic virus	2 min.	—	++	+	—	—	—
	2 hr.	—	++	++	++	++	++
	24 "	—	++	++	++	++	++
Cucumber virus 3	2 "	—	—	—	—	+	+
	24 "	—	—	—	+	++	++

2. *Cucumber Virus 3 as Antigen.*

Antiserum.	Time.	Dilution of antigen (1/1=1 mg. per c.c.).					
		1/1.	1/4.	1/16.	1/64.	1/256.	1/1024.
Tobacco mosaic virus	1 hr.	—	—	—	+	++	—
	24 "	—	—	—	++	++	++
	2 "	—	—	—	—	+	—
Aucuba mosaic virus	24 "	—	—	—	—	++	++
	2 "	—	—	—	—	+	—
	24 "	—	—	—	—	++	++
Enation mosaic virus	24 "	—	—	—	—	++	++
	2 min.	++	++	+	—	—	—
	2 hr.	++	++	++	++	++	++
Cucumber virus 3	24 "	++	++	++	++	++	++

In all tests the antiserum was used at a dilution of 1/50. 1 c.c. of antiserum was added to tubes containing 1 c.c. of antigen at given dilutions, and the tubes were immediately placed in a water-bath at 50° C.

+ signs indicate the degree of precipitation.

mosaic virus. Chester does not seem to have considered the possibility of quantitative antigenic differences and his tests seem to have been entirely qualitative; after the sera had been absorbed, tests for further precipitation were made at only one antigen or antiserum dilution. The tests in which no precipitation was obtained do not therefore necessarily indicate that the serum was completely absorbed, as Chester states, for they may have been made in a region of antigen excess which inhibited precipitation, as shown in Table II. Using purified virus preparations we have made cross-absorption experiments between our virus strains and their antisera, and we have found differences of the same type as those described by Chester. In these tests, as in the straightforward precipitation tests, much greater differences were found between the cucumber and the tobacco viruses than were found between the individual strains of the tobacco viruses. In each test a preliminary experiment was made to determine the optimal combining proportions of the antigen (virus) used for the absorption and the antiserum to be absorbed. The optimal combining proportions were determined by adding 1 c.c. of antigen at different concentrations to each of a series of tubes containing 1 c.c. of serum at a constant dilution. The tubes were immediately placed in a water-bath at 50° C. and the proportions of antigen and antiserum in that tube which first showed a precipitate were taken as optimal (Topley and Wilson, 1936, p. 144). Antigen and antiserum were then mixed with the antigen in slight excess of its optimal value; the mixture was then incubated for 2 hours at 50° C., placed in the ice-box overnight and then centrifuged. The supernatant fluid was then tested at a constant dilution against the antigen used for absorption at a number of different antigen dilutions. If there was no further precipitation it was then tested against the other virus strains, but if it still precipitated a second absorption was made. The sera, fully absorbed with one virus strain, were then tested for their ability to precipitate with the different virus strains, the tests being made with antiserum at a constant dilution and with antigen varying over a wide range of dilutions.

The amount of virus required to absorb an antiserum varied greatly with the different antigens and antisera: it was greatest when antigens were used to absorb their homologous antisera, somewhat less when the strains of tobacco mosaic virus were used to absorb each other's sera, and very much less when cucumber virus 3 was used to absorb tobacco mosaic virus antiserum or tobacco mosaic virus to absorb cucumber virus 3 antiserum. When tobacco mosaic virus antiserum was absorbed with cucumber virus 3 the precipitation of the serum with tobacco mosaic virus was only slightly affected, and the optimum precipitation point was only slightly shifted. Similarly, when cucumber virus 3 antiserum was completely absorbed with any of the three tobacco mosaic virus strains, its precipitation with cucumber virus 3 was only slightly affected. On the other hand, when antiserum to one strain of tobacco mosaic virus was fully absorbed with another strain its precipitation with the strain used for immunization was greatly affected, and the optimum precipitation point showed a large shift.

In Table III are shown the summarized results of several experiments in which the various antisera were absorbed with different virus strains and then

TABLE III.—*Summarized Results of Cross-absorption Experiments.*

Antiserum absorbed.	Antigen used for absorption.	Precipitation tests with absorbed sera and antigens.			
		Antigen.			
		Tobacco mosaic virus.	Aucuba mosaic virus.	Enation mosaic virus.	Cucumber virus 4.
Tobacco mosaic virus	(T.M.V.	—	—	—	—
	A.M.V.	—	—	—	—
	E.M.V.	++	++	—	—
	C.V. 3	++	++	++	—
	C.V. 4	++	++	++	—
	T.M.V.	—	++	++	—
Aucuba mosaic virus	(A.M.V.	—	—	—	—
	E.M.V.	++	++	—	—
	C.V. 3	++	++	++	—
	C.V. 4	++	++	++	—
	T.M.V.	—	+	++	—
	A.M.V.	—	—	+	—
Enation mosaic virus	(E.M.V.	—	—	—	—
	C.V. 3	++	++	++	—
	C.V. 4	++	++	++	—
	T.M.V.	—	+	++	—
	A.M.V.	—	—	+	—
	E.M.V.	++	++	++	—
Cucumber virus 3	(C.V. 3	++	++	++	—
	C.V. 4	++	++	++	—
	T.M.V.	—	—	—	++
	A.M.V.	—	—	—	++
	E.M.V.	—	—	—	++
	C.V. 3

— indicates that there is no precipitation.

+ signs indicate the degree of precipitation at the optimum. For description of method see text.

tested for their precipitability with other virus strains. From these it is apparent that tobacco mosaic antiserum absorbed with *Aucuba* mosaic virus contains no residual precipitating antibodies, while when absorbed with *Enation* mosaic virus it still precipitates with *Aucuba* mosaic and tobacco mosaic viruses; *Aucuba* mosaic virus antiserum after absorption with *Enation* mosaic virus still precipitates with both tobacco mosaic and *Aucuba* mosaic viruses, and after absorption with tobacco mosaic virus it still precipitates with *Enation* mosaic and *Aucuba* mosaic viruses; *Enation* mosaic virus antiserum after absorption with *Aucuba* mosaic virus will still give a slight precipitate with *Enation* mosaic virus but none with tobacco mosaic virus, and after absorption with tobacco mosaic virus it precipitates with both *Aucuba* mosaic and *Enation* mosaic viruses.

The results show that in addition to an antigenic fraction common to the three strains of tobacco mosaic virus examined, tobacco mosaic virus and *Aucuba* mosaic virus contain a fraction not present in *Enation* mosaic virus, *Aucuba* mosaic virus and *Enation* mosaic virus contain a fraction not present in tobacco mosaic virus, and *Enation* mosaic virus contains a fraction not present in *Aucuba* mosaic virus. If each fraction distinguished be represented by a letter, then the simplest formulæ for the three strains which can adequately explain the results are :

Tobacco mosaic virus A B.
Aucuba mosaic virus A B C.
Enation mosaic virus A — C D.

It is to be expected that the use of a larger number of strains in the cross-absorption experiments would have shown further differences between the three strains, and it is possible that each of the symbols in the formulæ represent groups of antigens rather than single antigens. If we assume that each of the components has an antigenicity of the same order as the others it would appear that the common fraction A is predominant; for removal of the antibodies to A greatly reduces the power of an antiserum to precipitate. There is, however, no definite evidence on the relative quantities of the different antigens present in these strains.

When tobacco mosaic virus antiserum is absorbed with either *Enation* mosaic or *Aucuba* mosaic virus it loses its power of precipitating with cucumber virus 3. The antigens which tobacco mosaic virus shares with cucumber virus 3 therefore must be contained in the common fraction A. The serological relationship between tobacco mosaic virus and cucumber virus 3 is best explained by postulating that, in addition to the antigens specific to each, the two viruses have two common antigens, and that while the total of the two antigens in each is of the same order, tobacco mosaic virus contains a preponderance of one and cucumber virus 3 a preponderance of the other. If we call the common antigens X and Y, then tobacco mosaic virus might be indicated as (NX nY) and cucumber virus 3 (nX NY). The antibody response in the rabbit will probably not be directly proportional to the amount of each antigen present, but the quantitative difference between the two components of the antiserum will be smaller than that between the two components of

VIRUS-CLUPEIN PRECIPITATES.

We have already shown (Bawden and Pirie, 1937*b*) that a paracrystalline precipitate, closely resembling that obtained with acid or ammonium sulphate, develops when neutral solutions of clupein and tobacco mosaic virus are mixed, and that the solubility of the precipitate in salt solution varies with the different virus strains. This phenomenon has now been investigated more fully, and certain similarities and differences in the behaviour of the three tobacco and two cucumber viruses have been determined. The precipitation appears to occur immediately the virus and clupein are mixed; it is greatly affected by changes in pH value and salt concentration, but is unaffected by small variations in temperature.

In the experiments described the amount of purified virus used was constant at 1 mg., while the other components of the system were varied and the final volume of the mixtures was 3.5 c.c. Readings of the extent of the precipitation were made by measuring the opacity of the suspensions with a photoelectric cell. When mixed with neutralized solutions of clupein sulphate (B.D.H.) none of the viruses gave a perceptible precipitate with 0.006 mg., and all gave maximum opacity with 0.05 mg. On the basis of their behaviour with intermediate quantities of clupein sulphate it was possible to divide the viruses into two groups; for tobacco mosaic and Enation mosaic viruses are more easily precipitable than Aucuba mosaic virus or either of the cucumber viruses: for example, with 0.04 mg. both tobacco mosaic and Enation mosaic viruses developed rather more than 50 p.c. of the maximum opacity, whereas the other three developed only 10 p.c.

Much more striking differences are obtained by varying the pH values and the salt contents of the mixtures, and these effects lead to a much better defined division of the viruses into two groups. When a system containing 0.06 mg. of clupein sulphate and 1 mg. of virus in 3.5 c.c. is studied, it is found that the precipitates with Enation mosaic and tobacco mosaic viruses are less soluble in the presence of salt than the precipitates with the other three viruses. For example, at pH 5.5 a suspension of the clupein compounds of Enation mosaic or tobacco mosaic virus in $M/60$ phthalate buffer has only half the opacity of a similar suspension in water, whereas with the other three viruses it is only necessary to raise the salt concentration to $M/200$ at this pH to produce this reduction in opacity. On either side of pH 5.5 the precipitates with all 5 viruses become less soluble in salt, and approximately twice the concentration is necessary to reduce the opacity to half at pH 5 or 6; thereafter the solubility rises again. The exact behaviour depends to a slight extent on the clupein-virus ratio, the strain of virus used and the particular salt; it is therefore unprofitable to attempt at present to give any great precision to a description of the system. It is clear, however, that under similar conditions the solubility minima have nearly the same pH values for all five viruses.

How far these results are generally applicable to viruses is unknown, but purified preparations of potato virus "X", which are also liquid crystalline, give with clupein sulphate an amorphous precipitate which is presumably

analogous to the para-crystalline precipitates described here. In interpreting histological appearances of virus-infected plants it is important that the existence of these complexes should be realized, for they are insoluble under conditions which may exist in the interior of cells, and similar virus precipitates are formed with some histones and protamines other than clupein.

It is well known that plants infected with strains of tobacco mosaic virus contain intracellular inclusions of two types. The one consists of rounded vacuolate bodies (X-bodies) and the other of flat plates. Iwanowski (1903) showed that the latter become striated when made acid, and Goldstein (1926) confirmed this, and stated that when acidified they seemed to be made up of distinct rods or needle crystals. Beale (1937) has pointed out that the needles obtained by acidifying these plates closely resemble the "crystals" described by Stanley (1936) in his acidified tobacco mosaic virus preparations, and suggests that the plates are the source of the virus. Examined between crossed Nicol prisms the plates are seen to be birefringent when viewed edge-ways but not when viewed flat. As many of the plates are definitely hexagonal this suggests that they may be true hexagonal crystals, for these are birefringent only when viewed along the transverse axes. Highly purified preparations of all five viruses give birefringent solutions, jellies and paracrystals, but no true crystals comparable to those seen in plants infected with strains of tobacco mosaic virus have yet been prepared. If, as the evidence suggests, the hexagonal crystals are depositions containing virus, there are several possible explanations for the different behaviour in the plant and after isolation. In the plant a process of slow crystallization which has not been simulated *in vitro* may go on; or the plates may be composed of a virus-host complex similar to the precipitates we have described with clupein; or the different crystalline states may have their origin in the different physical states of the virus before and after isolation. We have previously produced evidence indicating that during the process of purification the virus undergoes an irreversible aggregation, and it is possible that small units as they occur in the plant can arrange themselves in true crystals, whereas the larger aggregates in the isolated virus preparations cannot. The X-bodies are apparently quite different structures from these plates, for they are not birefringent, and Beale (1937) has shown that they are unaffected by acid.

In cucumber plants infected with viruses 3 and 4 no intracellular inclusions have been seen, and if they occur it must be much more rarely than in solanaceous plants infected with the strains of tobacco mosaic virus. The source of the virus therefore cannot lie entirely in the inclusions, as Beale suggests, but the absence of crystals from infected cucumbers might lend support to the idea that their production is a function of the concentration of virus; for, as we have stated above, the yield of cucumber viruses per volume of expressed sap is much less than that of the tobacco mosaic viruses. Other factors, however, might equally well explain the absence of inclusions: for example, the sap of cucumbers is very much more alkaline than is that of solanaceous plants, and this would increase the solubility of either the viruses or of virus complexes of the type we have described.

DISCUSSION.

It has been realized for some years that certain plant viruses occur in numerous strains: in general the strains possess similar host ranges and properties, but are differentiated because they cause different symptoms. A considerable amount of circumstantial evidence has also been accumulated, indicating that strains are continuously arising by a process analogous to mutation. Various workers have shown that recognized virus strains are closely related serologically, and that they have the power of immunizing plants against one another. A relationship of the type that exists between the tobacco mosaic viruses and cucumber viruses 3 and 4 does not seem to have been described before. Cucumber viruses 3 and 4 were recognized to be related strains, but because of their different host range they had not previously been thought to be related to tobacco mosaic virus. From our results it is apparent that the five viruses studied fall into one main group; the analytical figures for all are so similar that they afford no differentiation, and the differences in the physical and chemical properties yet found are on the whole trivial. They are sufficient, however, to show that the nucleoproteins isolated from plants infected with strains differentiated on phytopathological grounds are different proteins. They also show that the greater the differences in host range and symptoms caused, the greater the differences that can be detected in the properties of the isolated viruses, and suggest the advisability of differentiating and grouping viruses by other than usual phytopathological methods. Of these, the serological technique and X-ray analysis would appear to be most useful. Straight precipitation tests are sufficient to distinguish between the tobacco mosaic viruses and the cucumber viruses, but to distinguish between the individual strains of tobacco mosaic virus the more sensitive cross-absorption test must be used. The X-ray measurements (Bernal and Fankuchen, 1937) show differences of the same order: all five viruses pack in the same manner, indicating that they are of the same general shape, but measurements of the main spacings are sufficient to distinguish between the tobacco mosaic viruses and the cucumber viruses, the latter having a smaller cross-section. The three strains of tobacco mosaic virus all give the same main spacings, but a consideration of the relative intensities of all the lines on the X-ray plate separates each strain with certainty from the others.

The relationship of cucumber viruses 3 and 4 to the tobacco mosaic viruses is difficult to define. It was suggested (Bawden, 1934) that, in discussing the potato virus "X" group, relationships are found analogous to those indicated by genera, species and varieties. If this view be adopted here then all five viruses examined could be regarded as belonging to one genus, cucumber viruses 3 and 4 as varieties of one species and the three strains of tobacco mosaic virus as varieties of a second species of the same genus.

Most plant viruses are not serologically related to tobacco mosaic virus and possess quite different properties *in vitro*. The fact that the five viruses described form such an uniform group gives us no reason to imagine that all other plant viruses are necessarily similar in their chemical properties.

SUMMARY.

Methods are described for the isolation of nucleoproteins from cucumber plants infected with cucumber viruses 3 and 4. These have not been isolated from uninfected plants, and all the available evidence indicates that they are the viruses themselves. Infections were obtained with 10^{-10} g., and specific precipitates with antiserum with $1/8 \times 10^{-6}$ g. Concentrated solutions are spontaneously birefringent and dilute solutions show anisotropy of flow: when sedimented by high-speed centrifugation they form birefringent jellies, and when precipitated with acid or ammonium sulphate they form needle-shaped para-crystals. Although these viruses have a distinct host range from tobacco mosaic virus, the purified preparations have similar chemical compositions and many properties in common with purified preparations of strains of tobacco mosaic virus; they differ from tobacco mosaic virus, however, more widely than the recognized strains of tobacco mosaic virus differ from each other. The cucumber viruses and the tobacco mosaic viruses have common antigens: the results of cross-absorption experiments between the various viruses and their antisera are described, and provisional antigenic formulæ suggested. Possible methods of relating and distinguishing between viruses and the relationship between the cucumber and tobacco viruses are discussed.

We have great pleasure in thanking Dr. G. C. Ainsworth for supplying us with cucumber viruses 3 and 4, Mr. E. T. C. Spooner for preparing the antisera used, and Prof. A. A. Miles for suggesting the quantitative interpretation of our serological results.

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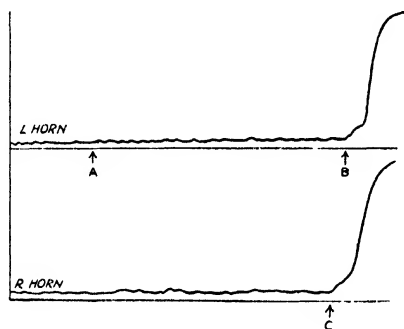
A NOTE ON ANAPHYLAXIS WITH TOBACCO MOSAIC VIRUS PREPARATIONS.

Chester (1936) showed that normal tobacco protein is strongly anaphylactogenic but that tobacco mosaic virus is not, for guinea-pigs sensitized with sap from infected plants could be desensitized completely with the sap

from healthy plants of the same species. Chester tested several preparations of tobacco mosaic virus prepared by Stanley, and although he found that they were not active anaphylactogens, yet they all reacted with guinea-pigs sensitized with healthy tobacco sap. Because of this Chester stated that the preparations of tobacco mosaic virus then available (1936) contained demonstrable amounts of normal plant proteins.

Dr. K. S. Chester has kindly tested one of our highly purified spontaneously birefringent solutions of tobacco mosaic virus which had been subjected in the course of the preparation to treatment with trypsin (Bawden and Pirie, 1937). This is the only preparation in which he has been unable to demonstrate the presence of normal protein by the anaphylactic test.

Thirteen pigs were injected by Dr. Chester with doses varying from 0.1 to 10.0 mg. of our preparation. The uterine horns from these pigs gave no



Kymograph tracing with the two horns of the uterus of a guinea-pig sensitized with 1 mg. of normal tobacco protein 19 days before. At A, 10 mg. of our preparation was added, at B 1.5 mg. of normal tobacco protein, and at C, 5 mg. of a preparation made by Stanley's technique.

reaction with normal tobacco protein or with tobacco mosaic virus preparations made by us or in the U.S.A. by Stanley's technique.

Six guinea-pigs sensitized with 1 mg. of normal tobacco protein reacted strongly both with tobacco protein (1.5 mg.) and with tobacco mosaic virus (5 mg.) preparations made by Stanley's methods, involving repeated "recrystallizations" and ultracentrifuging twice, but they gave no reaction with 10 mg. of our product. The results of one of these tests are shown in the figure.

We have previously suggested that the needles precipitated from solutions of tobacco mosaic virus by means of acid or ammonium sulphate are not true crystals, and that there is no reason to assume that preparations are necessarily pure because their properties are unaffected by repeated "recrystallization". Dr. Chester's results show quite clearly that incubation with trypsin readily effects a fractionation that cannot always be obtained by precipitation methods or by centrifugation.

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Brood Diseases of the Bee

by H. L. A. Tarr-Harpenden, Herts.

I. Distribution of the various diseases of the brood in different countries

In many countries no accurate information with reference to the prevalence of the different brood diseases is available. No attempt has been made to construct exhaustive records of the distribution of these diseases in a very large number of countries, the information given in the following tables having been obtained from different localities in which accurate surveys have been carried out.

1. Switzerland (Morgenthaler ¹⁴⁻³¹).

Disease	Cause	Number of samples received in the year recorded below									
		1917	1918	1919	1920	1921	1922	1923	1924	1925	1926
American foul brood (Bösartige Faulbrut).	Caused by <i>Bacillus</i> larvae White ^{53, 46}).	34	34	58	96	105	69	85	94	71	74
European foul brood (Gutartige Faulbrut).	Probably caused by <i>Bacillus pluton</i> White ^{52, 43, 45, 48}).	8	25	21	26	22	18	32	43	32	46
Sac brood (Sackbrut)	Caused by a filterable virus ⁽⁵¹⁾ .	—	13	15	9	22	12	28	37	20	10
Chalk brood (Kalkbrut).	Caused by a mould, <i>Pericystis apis</i> Maassen ^{12, 13}).	1	1	1	0	1	1	0	2	2	4
Stone brood (Steinbrut) (<i>Aspergillus</i> Mykose).	Caused by <i>Aspergillus flavus</i> .	0	0	0	0	0	0	0	0	0	0
Decomposing drone brood (Zersetzte Buckelbrut).	Caused by a drone-laying queen.	—	—	—	19	21	24	25	26	18	14
Uncertain or no disease (Keine oder Unbekannte Krankheit).	Causes unknown.	55	58	55	39	74	68	74	84	66	61

(Table continued).

Disease	Cause	Number of samples received in the year recorded below								
		1927	1928	1929	1930	1931	1932	1933	1934	1935
American foul brood (Bösartige Faulbrut).	Caused by <i>Bacillus</i> larvae White ^{53, 46}).	62	73	75	67	85	59	81	65	90
European foul brood (Gutartige Faulbrut)	Probably caused by <i>Bacillus pluton</i> White ^{52, 43, 45, 48}).	47	78	54	60	52	73	108	121	140
Sac brood (Sackbrut).	Caused by a filterable virus (⁵¹).	19	22	8	13	19	18	35	19	19
Chalk brood (Kalkbrut).	Caused by a mould, <i>Penicillium apis</i> Maassen ^{12, 13}).	2	4	4	6	7	7	5	8	8
Stone brood (Steinbrut) (<i>Aspergillus</i> Mykose).	Caused by <i>Aspergillus flavus</i> .	0	0	1	1	0	0	0	0	0
Decomposing drone brood (Zersetzte Buckelbrut).	Caused by a drone-laying queen.	24	32	29	32	22	25	39	45	39
Uncertain or no disease (Keine oder Unbekannte Krankheit).	Causes unknown.	59	73	80	91	116	120	135	120	122

2. Germany (Borchert ^{1, 2}).

The figures given in the following table include chiefly cases of brood disease found in the Provinces of: Preussen, Bayern, Sachsen, Thüringen, Württemberg, Baden, Hessen and Oldenburg.

Disease	Number of cases reported	
	1930	1931
American foul brood ..	1844	3996
European foul brood...	52	73
Chalk brood	30	24
Stone brood	9	—
Sac brood	60	22

3. England (Tarr ^{41, 42, 43, 48}).

Disease	Number of cases reported *)		
	1934	1935	1936
American foul brood	26	43	35
† Added brood	17	17	24
European foul brood	8	3	2
Sac brood	2	2	0
Chalk brood	2	4	5
Chalk brood and drone laying queen	1	1	1
Drone laying queen and decomposing brood	4	0	1
American foul brood and Added brood	—	1	0
American foul brood and Chalk brood	—	1	0
Chilled or neglected brood..	—	—	4
Spray poisoning (?)	—	—	1
Totals	60	72	73

*) The figures given only represent samples voluntarily sent to the laboratory for diagnosis.

†) This disease has been described elsewhere (Tarr ⁴⁷). It is possible that some of the cases reported by Morgenthaler as „Keine oder Unbekannte Krankheit” were this type of disease.

4. The United States of America.

A record of samples of brood disease received at the Bee Culture Laboratory, Washington, U.S.A. from 1906 to 1925. (Sturtevant ^{36a}).

Year	Disease							
	American foul brood	American foul brood (?)	European foul brood	European foul brood (?)	Sac brood	Sac brood (?)	Mixed *) infection	Uncertain †)
1906	8	3	19	9	11	—	—	15
1907	46	—	29	5	13	—	—	30
1908	18	—	6	4	5	1	—	37
1909	178	—	136	31	66	14	—	116
1910	242	—	126	38	58	16	—	134
1911	236	—	156	31	54	3	1	291
1912	108	—	145	19	74	10	—	258
1913	89	—	96	28	58	6	—	124
1914	73	—	96	19	27	12	—	80
1915	107	2	100	13	23	12	—	108
1916	123	2	66	15	20	14	2	82
1917	147	2	91	10	47	19	1	82
1918	189	—	95	5	34	11	2	78
1919	277	2	156	15	59	25	19	94
1920	321	—	159	2	79	16	14	117
1921	478	1	292	8	117	27	17	138
1922	394	1	147	3	98	17	2	146
1923	383	1	124	2	56	12	4	176
1924	307	1	124	2	43	5	(?) 1	118
1925	312	—	97	10	28	6	2	128
Total .	4036	15	2260	269	970	226	(?)64	2372

*) American foul brood and European foul brood occurring in the same comb.

†) Disease not definitely determined.

5. Canada (Gooderham ⁶, ^{6a}).

(Samples sent voluntarily to the Central Experimental Farm, Ottawa, for diagnosis).

Disease	No. of cases reported										
	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931 to 1933
American foul brood	—	8	25	24	8	22	48	27	16	39	125
European foul brood	—	7	16	6	9	28	27	21	17	39	29
Sac brood	—	10	0	3	0	0	0	0	0	0	0
No disease	—	1	9	5	2	11	17	12	7	18	89
Totals	20	26	50	38	19	61	92	60	40	96	243

6. The incidence of American foul brood and European foul brood in colonies of bees owned by members of the Vereins Deutsch-Schweizerische Bienenfreunde (V.D.S.B.) from 1908—1935*).

Year	Number of members	Number of colonies of bees	Number of colonies affected with American foul brood		Number of colonies affected with European foul brood	
			Total No. affected	% affected	Total No. affected	% affected
1908	7,035	88,741	93	1,33	3	0,04
1909	7,163	90,882	88	1,13	12	0,17
1910	7,498	102,197	61	0,83	7	0,09
1911	7,532	105,179	82	1,06	3	0,04
1912	8,740	115,206	62	0,71	6	0,07
1913	9,083	122,227	72	0,79	5	0,06
1914	9,186	118,059	49	0,53	10	0,11
1915	9,224	118,910	29	0,32	8	0,08
1916	9,649	122,596	24	0,25	7	0,07
1917	11,872	150,653	46	0,39	2	0,02
1918	13,660	146,103	51	0,38	7	0,05
1919	14,684	162,346	69	0,42	9	0,06
1920	15,734	165,923	98	0,62	6	0,04
1921	15,898	172,794	78	0,49	8	0,05
1922	15,785	173,158	59	0,38	10	0,06
1923	15,985	175,568	78	0,49	12	0,07
1924	16,295	184,535	84	0,52	13	0,08
1925	16,433	184,675	50	0,31	12	0,07
1926	16,534	180,267	37	0,23	20	0,12
1927	16,582	191,429	40	0,24	50	0,30
1928	16,414	185,024	73	0,44	61	0,37
1929	16,554	186,432	45	0,28	44	0,27
1930	16,952	196,766	50	0,30	38	0,22
1931	17,290	205,238	62	0,36	58	0,34
1932	17,477	205,912	20	0,23	73	0,42
1933	17,350	204,516	71	0,29	84	0,49
1934	17,904	210,351	72	0,33	88	0,48
1935	18,790	223,454	73	0,27	125	0,60
Totals			1,736	0,50	484	0,18

*) (See Leuenberger 8-11).

The above results expressed graphically (Fig. 1) show that American foul brood has been treated successfully in Switzerland while European foul brood has commenced to increase seriously (Morgenthaler³²), Leuenberger⁸).

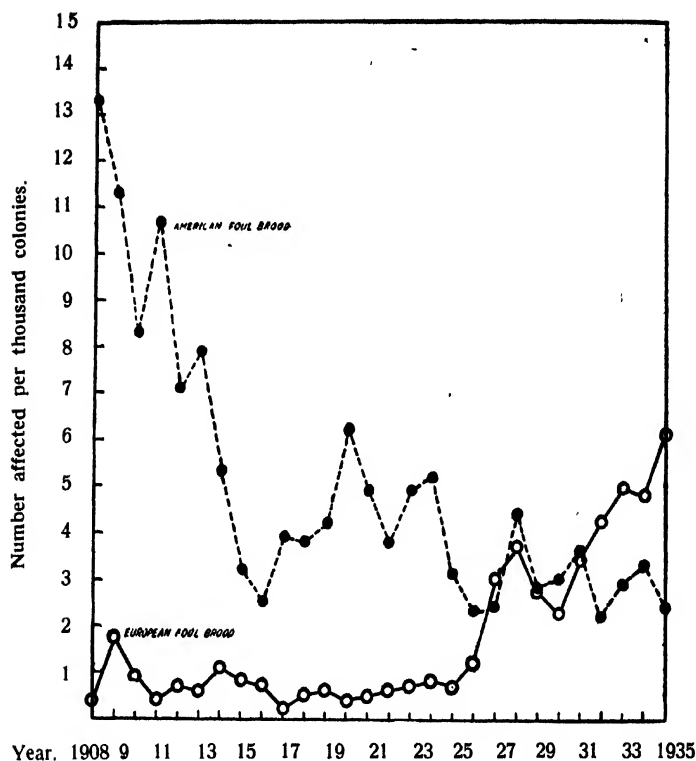


Figure 1.

7. Simultaneous occurrence of American and European foul brood in a single colony of bees.

American foul brood and European foul brood occasionally occur simultaneously in the same colony of bees: thus between 1905 and 1920 of 7568 samples of diseased brood comb received at the Bee Culture Laboratory, Washington, 38 were mixed infection.

(Sturtevant³⁷).

Year	Samples received containing both European and American foul brood	Number of samples received during the year given
1911	1	1042
1916	2	374
1917	1	449
1918	2	429
1919	18	693
1920	14	698

The seasonal distribution of the samples was as follows:

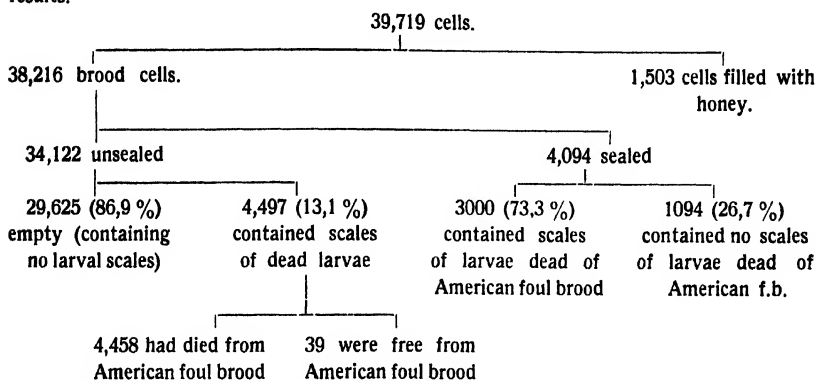
(Sturtevant³⁷).

Month	Number of samples
April	5
May	9
June	10
July	1
August	6
September	5
October	1
November	1

II. American foul brood

1. The distribution of the dry scales of larvae dead of American foul brood in the sealed and unsealed cells of brood combs (Borchert⁴, 5).

39,719 cells in 1,146 rows of cells on 30 surfaces of 16 different samples of brood comb containing dry scales of larvae dead of American foul brood were examined with the following results.



11.2 % of all cells examined were open (unsealed) cells containing scales of larvae dead of American foul brood.

7.6 % of all cells examined were sealed cells containing scales of larvae dead of American foul brood.

11.7 % of all brood cells examined were unsealed cells containing scales of larvae dead of American foul brood.

7.8 % of all brood cells examined were sealed cells containing scales of larvae dead of American foul brood.

13.1 % of all unsealed cells examined contained scales of larvae dead of American foul brood.

73.3 % of all sealed cells examined contained scales of larvae dead of American foul brood.

(A very large number of the cells containing the dead larvae are unsealed by the bees in their attempt to remove the scales).

2. The resistance of the spores of *Bacillus larvae* (the organism causing American foul brood) to heat, sunlight and certain disinfectants (White⁵³).

The thermal death point of spores of *B. larvae*, obtained from scales of dead larvae, when suspended in water.

Temperature °C.	Period of heating in mins.	Results shown by cultures (employing egg agar, White ⁵³)	
0	0	Numerous spores alive (check)	Material from America.
90	20	Many spores not killed	
94	10	One spore not killed	
96	10	All spores killed	
97	10	" " "	
98	10	" " "	
99	10	" " "	
100	5	" " "	
100	10	" " "	
100	10	" " "	

(Table continued).

Temperature °C.	Period of heating in mins.	Results shown by cultures (employing egg agar, White ⁵³)	
0	10	Numerous spores alive (check)	Material from England.
91	10	Spores not killed about 1/10 th. as many as in check	
95	10	All but two spores killed	
96	10	All spores killed	
98	10	" " "	
99	10	" " "	
100	1	Spores not killed about 1/4 as many as in check	
100	2	All but 100 spores killed	
100	3	" " 20 " "	
100	4	" " 20 " "	Material from France.
100	5	" " 20 " "	
0	10	Numerous spores alive (check)	
90	10	Spores not killed almost as many as in check	
92	10	Spores not killed about 1/4 as many as in check	
93	10	All but 30 spores killed	
94	10	" " 100 " "	
96	10	" " 12 " "	
98	10	" " 1 " "	
99	10	All spores killed	
92	10	Numerous spores not killed	Material from Cuba.
93	10	" " " "	
94	10	Fewer spores not killed	
95	10	" " " "	
96	10	" " " "	
97	10	Fewer alive than at 96°	
98	10	" " " " 97°	
99	10	About 12,000 spores not killed	
100	10	" 200 " " "	
100	11	" 200 " " "	
100	12	" 200 " " "	

Variations in the thermal death point of spores of *B. larvae* obtained from scales of dead larvae from different localities in the United States. (suspended in water). (White ⁵³).

Temp. °C.	Period of heating in mins.	Source of samples (State)										
		Wash.	Minn.	Nebr.	Ohio	Ill.	Col.	Wis.	Penn.	Penn.	Ohio	Mont
100	10	—	—	+	+	+	+	+	+	+	+	+
100	5	—	—	—	—	—	—	+	+	+	+	+
100	1	—	—	—	—	—	—	—	—	—	—	—
98	10	—	—	—	—	—	—	+	+	+	+	+
95	10	—	—	—	—	—	—	—	—	—	—	—

— = spores not all killed. + = spores all killed.

The reason for the variation in the thermal death point of spores of *B. larvae* from different localities is not known.

Resistance of the spores of *B. larvae* to a temperature of 100° C. when suspended in water (Cuban sample used). (White ⁵³).

Period of exposure in mins.	Results shown by cultures (employing egg agar) (White ⁵³).
0	25.000 spores alive (estimated).
1	4.000 " " "
5	148 " "
6	220 " "
7	248 " "
8	44 " "
9	7 " "
10	14 " "
11	All spores killed.
12	" " "
13	" " "
14	" " "
15	" " "

Resistance of spores of *B. larvae* to heat when suspended in undiluted honey. (White ⁵³).

Temp. in °C.	Period of exposure in mins.	Results shown by cultures (Employing egg agar, White ⁵³)	Origin of sample
100	10	Numerous spores not killed	Cuba
100	10	" " " "	Washington
100	10	" " " "	Ohio
100	20	" " " "	Cuba
100	20	" " " "	Washington
100	20	" " " "	Ohio
103	25	" " " "	Washington
105	20	" " " "	"
105	20	" " " "	Cuba
107	20	" " " "	"
107	30	" " " "	"
107	40	" " " "	"

Resistance of dried spores of *B. larvae* to the sun's rays. (White ⁵³).

Period of exposure in hrs.	Results shown by cultures (employing egg agar) (White ⁵³)	Period of exposure in hrs.	Results shown by cultures (employing egg agar, White ⁵³)
2	Numerous spores not killed	38	12 spores not killed
4	" " " "	28	All spores killed
5	" " " "	37	" " "
7	Several " " "	41	" " "
10	A few " " "	41	" " "
11	" " " "	44	" " "
12	Many spores not killed	61	" " "
29	Several spores not killed	79	" " "

Resistance of spores of *B. larvae* to the sun's rays when suspended in honey (White⁵³).

Period of exposure	Results of inoculating healthy colonies of bees				
6 hrs.	Heavy American foul brood infection				
13 "	Moderate	"	"	"	"
2 weeks	Slight	"	"	"	"
4 "	Considerable	"	"	"	"
5 "	"	"	"	"	"
4 "	No disease produced				
5 "	"	"	"	"	"
6 "	"	"	"	"	"
8 "	"	"	"	"	"

Resistance of spores of *B. larvae* to various chemical compounds when suspended in honey and fed to healthy colonies of bees (White⁵³).

Compound	Concentration	Results of inoculation			
β -naphthol	1 : 2000	American foul brood produced			
"	1 : 1000	"	"	"	"
"	1 : 500	"	"	"	"
Phenol	1 : 2000	"	"	"	"
"	1 : 1000	"	"	"	"
"	1 : 500	"	"	"	"
"	1 : 100	"	"	"	"
Oil of Eucalyptus	1 : 250	"	"	"	"
Formaldehyde	3 : 1000	"	"	"	"
Salicylic acid	1 : 2000	"	"	"	"
" "	1 : 1000	"	"	"	"
" "	1 : 500	"	"	"	"
Phenyl salicylate (Salol)	1 : 2000	"	"	"	"
" " "	1 : 1000	"	"	"	"
" " "	1 : 500	"	"	"	"
Quinine bisulphate	1 : 500	"	"	"	"
" "	3 : 1000	"	"	"	"
" "	1 : 100	"	"	"	"

The results given show that no protection against American foul brood is afforded by any of the chemical compounds listed.

3. Variations in the size of *B. larvae* spores (Borchert³).

Length in microns of 800 spores from 40 honey samples taken from foul brood combs		Length in microns of 40 spores in one honey sample containing added foul brood material		Length in microns of 300 spores from larvae dead of American foul brood	
5 spores	1,1	12 spores	1,3	4 spores	1,1
375 "	1,3	22 "	1,5	120 "	1,3
380 "	1,5	6 "	1,7	130 "	1,5
38 "	1,7			43 "	1,7
2 "	1,9			3 "	1,9
Average	1,4	Average	1,5	Average	1,4

4. The metabolism of *B. larvae* in relation to the stage at which larvae are affected with American foul brood.

The effect of various glucose concentrations on the germination of spores and the growth of vegetative cells of *B. larvae* on egg agar (Sturtevant³⁸)

Material studied	% of added glucose in the medium														
	0	0,5	0,7	1,0	1,3	1,5	1,75	2,0	2,25	2,5	2,75	3,0	3,5	4,0	4,5
Spores from larvae dead of American f.b.	++++	++	+++	++++	++++	+++	+++	++	+	+	+	+	G	G	G
Vegetative cells from pure cultures of <i>B. larvae</i>	++++	+++	+++	++++	++++	++++	+++	+++	++	++	++	++	+	+	±
+ = Slight growth ++++ = Heavy growth G = Slight germination of spores ++ = Fair growth +- = Doubtful growth - = No evidence of growth +++ = Good growth															

Average number of colonies developing from one 4 mm loopful of a vegetative cell culture of *B. larvae* in broth agar medium with various glucose concentrations (Sturtevant³⁸).

% of glucose in medium	Average number of colonies of <i>B. larvae</i> developing	% of glucose in medium	Average number of colonies of <i>B. larvae</i> developing
Control (no glucose)	1,500	2,0	—
0,5	1,590	2,5	150
1,0	1,560	3,0	0
1,5	914	3,5	0

Unassimilated reducing sugar in whole larvae of different ages (Sturtevant³⁸).

Age of larvae in days	Number of larvae examined	Avg. wt. of larvae examined in gms	Avg. wt. of reducing sugar per larva (calculated as glucose)	% of reducing sugar in each larva	Remarks
2	50	0,01247	0	0	Average age for sealing cells.
3	175	0,04322	0,000475	0,98	
4	525	0,10314	0,00299	2,82	
5	475	0,13591	0,00428	3,14	
6	150	0,15581	0,00372	2,39	
7	125	0,14397	0,00203	1,40	Average age at which death occurs from American foul brood.
8	50	0,13762	0	0	
9	25	0,13293	0	0	

The results set out in the above tables indicate that *B. larvae* will not multiply when the reducing sugar concentration rises much above 2,5—3 %, and it has been suggested that American foul brood normally affects larvae about 8 days of age because at this stage the reducing sugar concentration in them is negligible. Recent experiments by the reviewer have shown that spores of *B. larvae* will germinate readily and the vegetative cells arising therefrom will grow in 12,5 % reducing sugar in a medium containing minced chicken embryo. The significance of this finding has been discussed elsewhere (in press).

culture media and to produce American foul brood in healthy colonies of bees.

(12 cc ph 6,8) in relation to the period of incubation at 37° C. (Sturtevant ³⁹).

Number of positive cultures obtained after the following number of days of incubation														Number of cultures		
17	18	19	20	21	22	23	24	25	26	27	28	29	30	Posi- tive	Nega- tive	Total
—	—	—	—	—	—	—	—	—	—	—	—	—	—	16	0	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	16	0	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	16	0	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	16	0	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	16	0	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	16	0	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	14	0	14
—	—	—	—	—	—	—	—	—	—	1	—	—	—	10	0	10
—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	2	10
—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	4	9
—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	2	10
—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	7	15
1	—	—	—	—	—	—	—	—	—	—	—	—	—	8	4	12
—	—	—	—	—	—	—	—	—	—	2	—	—	—	11	2	13
1	—	—	1	—	—	—	—	—	—	1	—	—	—	7	9	16
—	—	—	—	1	—	—	—	—	—	—	—	—	—	4	10	14
—	—	—	—	—	—	—	—	1	—	—	—	—	—	4	10	14
—	—	—	1	1	1	—	—	—	—	1	—	—	—	5	10	15
—	—	—	—	—	—	—	—	—	—	1	—	—	2	6	7	13
—	—	—	—	—	—	—	—	—	—	1	—	—	—	3	12	15
—	—	—	—	—	—	—	—	—	—	2	—	—	—	2	13	15
—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	15	15
—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	15	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	15	16
—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	15	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	15	16
—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	14	16
—	—	—	—	—	—	—	—	—	—	2	—	—	—	3	12	15
—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	15	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	14	16
—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	123	123
2	0	0	2	2	1	0	0	1	0	12	0	0	4	211	—	—
367	367	367	365	363	362	362	362	361	361	349	349	349	345	—	345	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	556

The number of spores of *B. larvae* required to produce American foul brood in healthy colonies of bees (Sturtevant 35)). (In this experiment duplicate colonies of bees (A and B) were used during the first five years, and triplicate colonies (A, B and C) during the last year. The spores fed were obtained from the scales of larvae dead of American foul brood, the inoculum for each colony being suspended in 1 litre of sugar syrup).

Inoculum (No. of spores fed)	First year		Second year			Third year		Fourth year			Fifth year			Sixth year		
	A	B	During season		Final inspection	Expt. repeated		During season		Final inspection	During season		Final inspection	During season		Final inspection
3000 × 10 ⁶	+	?	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2500 × 10 ⁶	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1000 × 10 ⁶	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
750 × 10 ⁶	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
500 × 10 ⁶	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
350 × 10 ⁶	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
200 × 10 ⁶	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
175 × 10 ⁶	—	—	?	+	—	—	—	—	—	—	—	—	—	—	—	—
150 × 10 ⁶	—	—	?	+	—	—	—	—	—	—	—	—	—	—	—	—
125 × 10 ⁶	—	—	?	+	—	—	—	—	—	—	—	—	—	—	—	—
100 × 10 ⁶	0	0	?	+	—	—	—	—	—	—	—	—	—	—	—	—
75 × 10 ⁶	—	—	?	+	—	—	—	—	—	—	—	—	—	—	—	—
50 × 10 ⁶	0	0	?	+	—	—	—	—	—	—	—	—	—	—	—	—
25 × 10 ⁶	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10 × 10 ⁶	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5 × 10 ⁶	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2.5 × 10 ⁶	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.5 × 10 ⁶	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.5 × 10 ⁶	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.1 × 10 ⁶	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
† Controls..	1 +	12—0	1 +	2—0	1 +	2—0	1—0	1 +	3—0	1 +	3—0	8—0	8—0	2—0	2—0	2—0

† Controls.. +, American foul brood. ? +, Probable American foul brood, very slight and unconfirmed and disappearing toward end of brood-rearing season. 0, No disease found during the season. — * American foul brood cleaned out by the end of the brood-rearing season. — **, No recurrence of American foul brood during the second season in which the experiment was carried out. †, The controls were healthy uninoculated colonies kept in the same apiaries as the inoculated ones.

The relative pathogenicity of endospores and vegetative cells of *B. larvae* for healthy colonies of bees (Tarr ⁴⁶).

(In these experiments both spores and vegetative cells of *B. larvae* were prepared from pure cultures of the organism employing the same culture medium).

Inoculum	Method of introducing the bacteria into the colony of bees	Results of the experiment
$8,4 \times 10^{10}$ vegetative cells of <i>B. larvae</i>	The bacilli were sprayed over the developing brood after suspending in 20 cc of broth.	No disease produced by the end of the brood-rearing season.
$17,2 \times 10^{10}$ vegetative cells of <i>B. larvae</i>	The bacilli were sprayed over the developing brood after suspending in 25 cc of broth.	" "
$6,2 \times 10^6$ spores.....	20 ml of an aqueous suspension sprayed over the developing brood.	" "
62×10^6 spores.....	" "	American foul brood produced.
620×10^6 spores.....	" "	" "
6200×10^6 spores.....	" "	" "
62×10^6 spores.....	Fed to the bees in 100 cc of sugar syrup.	No disease produced by the end of the brood-rearing season.
620×10^6 spores.....	" "	" "
6200×10^6 spores.....	" "	American foul brood produced.
62000×10^6 spores.....	" "	" "

Toumanoff ⁴⁹) found that not one of 302 one to five day old bee larvae individually inoculated with a small drop of heavy aqueous suspensions of 48—92 hour old egg agar cultures of *B. larvae* (chiefly vegetative cells of the organism) developed American foul brood. 170 of these larvae were removed by the bees, and the remainder (132) matured normally into adult bees. Sturtevant ³⁹) found that at least 10 million spores of *B. larvae* in 0,1 cc of syrup are required to produce American foul brood when fed directly to 4 day old larvae.

The following inferences may be drawn from the above data:

1. At least 50,000 spores of *B. larvae* are required to initiate vegetative growth of this organism under the experimental conditions described.
2. Healthy colonies of bees will not develop American foul brood unless they receive at least 50×10^6 spores of *B. larvae* in 1 litre of syrup.
3. Vegetative cells of *B. larvae* are apparently not pathogenic for the brood of healthy colonies of bees even when introduced in doses some 3000 times greater than an inoculum of spores of the organism sufficient to produce American foul brood.
4. Smaller inocula of *B. larvae* spores produce disease in healthy colonies of bees when the brood is sprayed directly with aqueous suspensions of them than when they are fed in syrup to the bees.

5. Individual larvae are infected only with relatively large doses of spores of *B. larvae* and are apparently not infected by feeding them vegetative cells of the organism directly.

6. The occurrence of spores of *B. larvae* in commercial honey.

The presence of spores of *B. larvae* in commercial honey samples in relation to their ability to produce American foul brood in healthy colonies of bees (Sturtevant ³⁹, ⁴⁰).

Source of sample	Number of samples examined	Number of samples showing spores resembling those of <i>B. larvae</i>	Number of samples showing no evidence of spores	Results of inoculating healthy colonies of bees (Sturtevant ⁴⁰)
Commercial honey .	187	15	172	Only 1 of the 15 samples containing <i>B. larvae</i> spores produced American foul brood in healthy bee colonies.
Samples from an exptl. apiary ...	2	0	2	
Miscellaneous	2	2	0	
Totals	191	17	174	

In these experiments the method of demonstrating spores was sufficiently accurate to estimate considerably less than 50×10^6 spores per litre of honey (the minimum lethal dose for an average colony of bees; vide supra). It has therefore been inferred that there are rarely sufficient spores of *B. larvae* in commercial honey samples to cause American foul brood.

The determination of *B. larvae* spores in honey in relation to the accuracy of the method employed (Sturtevant ⁴⁰).

(Spore counts were made of stained smears of the sediments resulting from centrifuging duplicate 5 cc. portions of 5 samples of honey containing known numbers of *B. larvae* spores).

Spore counts in duplicate samples (A and B) containing the following numbers of spores per cc.

	50,000		300,000		500,000		800,000		1,000,000	
	A	B	A	B	A	B	A	B	A	B
Total count of 30 fields	38	39	233	244	393	400	638	641	791	835
A + B	77		477		793		1,279		1,626	
Actual mean no. of spores per field recovered	1.2833		7.9500		13.2167		21.3167		27.1000	
Theoretical recovery	1.5205		9.1230		15.2050		24.3279		30.4100	
Ratio of actual mean to theoretical mean (% recovery)	84.40		87.14		86.92		87.62		89.12	

From this data, using the method employed by Sturtevant for demonstrating and counting spores of *B. larvae* in honey, the number of spores in an unknown sample of honey can be estimated from the mean number of spores in 60 microscopic fields as follows:

$$\bar{X} = 1.1228Y + 0.2034 \times 32,884$$

where: Y = The mean number of spores in 60 fields (duplicate samples).

\bar{X} = The theoretical number of spores that should be recovered if no loss occurs.

and 32,844 = the constant (for the method used) for converting the theoretical number of spores per field to spores per cc of the unknown honey sample.

7. The disinfection of American foul brood combs.

Although much data with reference to the sterilization or disinfection of American foul brood combs has been published (Sturtevant³⁶) no really satisfactory method has been evolved (Hambleton^{6b}). At the present time the consensus of opinion is that disinfection is neither satisfactory nor economical, and for this reason no attempt to record the data will be made here.

8. The natural resistance of colonies of bees to American foul brood.

Although American foul brood is normally a fatal disease, the affected colony succumbing to the infection, in rare instances infected colonies of bees have been known to recover from the disease. This fact has led to the initiation of important experiments in the United States as a result of which it is hoped that eventually a race of bee naturally resistant to this destructive disease will be evolved. Colonies of bees which have been reported as possessing a marked resistance have been segregated in an isolation apiary in order that breeding experiments may be carried out, and it is hoped that the factors responsible for resistance to American foul brood will be successfully perpetuated in an „American foul brood resistant” strain of bee. The following table records the results of preliminary experiments.

The results of inoculating presumed „American foul brood resistant” and „American foul brood non-resistant” (controls) colonies of bees with combs containing scales of larvae dead of American foul brood (Park, Pellett and Paddock³³).

Number of colonies of bees inoculated	Number of colonies of bees free from American foul brood by the end of the brood-rearing season	
	First year of expt.	Second year of expt.
First year of expt.		
Colonies assumed to be non-resistant to American foul brood 6	0	0
Colonies assumed to be resistant to American foul brood 25	7 (28 %)	12 (48 %)
Second year of expt.		
Colonies assumed to be non-resistant to American foul brood 2	—	0
Colonies assumed to be resistant to American foul brood:		
*Second generation 27	—	9 (33 $\frac{1}{3}$ %)
Other sources 7	—	5 (71 %)
—	—	—
34	—	14
Totals for the two years:		
Assumed non-resistant 8	—	0
„ resistant 59	—	26 (44 %)

*) The original queens had superseded in the apiary.

9. The efficiency of the „Shaking Treatment“ (Artificial Swarm) in eradicating American foul brood from affected colonies of bees.

Dunham and King ^{5b}) record the results obtained by practical beekeepers using the shaking method in an attempt to eradicate American foul brood from affected colonies of bees. 18 different beekeepers treated a total of 300 colonies in their apiaries by the usual methods: of these 300 colonies 81 developed the disease again, 56 during the first season following treatment and 25 during the second season following treatment.

The effect of treating colonies affected with American foul brood under experimental conditions (Dunham and King ^{5b})).

Method of treating	Number of colonies treated	Number of colonies developing American foul brood during the two years after treatment
Empty hive plus foundation	18	1
Foundation	18	1
Foundation plus drawn comb	17	2
Drawn comb	18	8

10. The pathological changes occurring in larvae affected with American foul brood: a comparison of the tissues and cells of diseased and healthy larvae of different ages.

The measurements given in the following tables were made on fixed and sectioned material obtained from artificially infected larvae (unless otherwise stated) of known ages.

A comparison of the lengths of normal healthy larvae with those of larvae affected with American foul brood (pathological) (Jaeckel ⁷)).

Healthy larvae		Pathological (affected with American foul brood)	
Age of larvae in days	Length of larvae in mm	Age of larvae in days	Length of larvae in mm
2	1,87— 2,5	2½	3,7— 4,0
3	4,3 — 5,9	3	2,0— 6,5
4	6,2 — 8,0	4	4,4— 7,8
5	7,5 — 8,0	5	7,3— 9,0
6	8,0 —11,5	6	6,1—10,0
7	12,0 —14,0	7	12,0—14,0 (coiled 7,7—8,5)
8	14,0 —16,0	8	14,0—15,0

A comparison of the size of various cells in healthy larvae and in those affected with American foul brood (Jaeckel ⁷)).

Cells measured	Size of cell in microns		Age of larvae (days)
	Normal (healthy)	Pathological	
Oenocytes	33	39	2
Nucleus of oenocytes	12	18	„
Cells of mid intestine (height) ...	15	33	„
(width)	18	30	„
Nucleus of cells of mid intestine..	14	27	„
Fat cells	15—20	12—15	„
Nuclei of fat cells	8— 9	9—10	„

(Table continued)

Cells measured	Size of cell in microns		Age of larvae (days)
	Normal (healthy)	Pathological	
Ganglion cells	5-6	5-8	2
Excretal cells	12-14	18-24	"
Nuclei of excretal cells	3	6-8	"
End cells of trachea (nuclei)	12	12-14	"
Malpighian tube cells:			
Length	3	4	"
Width	8-10	8-10	"
Nuclei	6-7	9-12	"
Hypodermal cells:			
Length	3-4	3-4	"
Width	4-5	4-5	"
Nuclei	3	3	"
Nuclei of cells of tracheal epithelium	4-6	5-7	"
Nuclei of muscle cells	6-8	16-20	"
Oenocytes	39	39	3
Nuclei of oenocytes	15	12-15	"
Cells of mid intestine:			
Length	20-40	39-45	"
Width	18-30	—	"
Nuclei	18	21-26	"
Fat cells	18-24	12-22	"
Nuclei of fat cells	9-11	9-12	"
Ganglion cells	6-7	6-7	"
Excretal cells	25-30	45-48	"
Nuclei of excretal cells	6-8	9-11	"
Nuclei of end cells of trachea	12-13	12-15	"
Malpighian tube cells:			
Length	} As in 2 day old larvae	15-19	"
Width		35-40	"
Nuclei		25-27 (long)	"
Hypodermal cells:			
Length	4-5	5-6	"
Width	4-6	5-6	"
Nuclei	4	5	"
Nuclei of cells of tracheal epithelium	4-6	6	"
Nuclei of muscle cells	6-9	12-18	"
Oenocytes	30-40	45	4
Nuclei of oenocytes	15-18	15-18	"
Cells of mid intestine	As in 3 day old larvae	Not measurable	"
Fat cells	30-40	18-25	"
Nuclei of fat cells	12	12	"
Ganglion cells	6-8	6-7	"

(Table continued)

Cells measured	Size of cell in microns		Age of larvae (days)
	Normal (healthy)	Pathological	
Hypodermal cells:			
Length	5—6	5—6	4
Width	5—6	5—6	"
Nuclei	4	5	"
Nuclei of muscle cells	10—15	12—14	"
(The figures given for all 5 day old larvae were from naturally infected ones).			
Oenocytes	45	39	5
Nuclei of oenocytes	18	24	"
Cells of mid intestine:			
Length	36	30	"
Width	15—18	—	"
Nuclei	15—18	33 (long)	"
Fat cells	36—40	40—45	"
Nuclei of fat cells	12—15	15—18	"
Ganglion cells	9—10	9—10	"
Hypodermal cells:			
Length	5—6	10—12	"
Width	5—6	6	"
Nuclei	4—5	5	"
Malpighian tube cells:			
Length	45—50	27	"
Width	36—38	—	"
Nuclei	21—24	18—24	"
Nuclei of muscle cells	15	15—18	"
Oenocytes	45—75	70—75	6
Nuclei of oenocytes	15—18	25—27	"
Cells of mid-intestine:			
Length	36	60	"
Width	36	—	"
Nuclei	18	30	"
Fat cells	39—50	24—30	"
Nuclei of fat cells	12—15	12—15	"
Excretal cells	40—45	20—60	"
Nuclei of muscle cells	12—15	12—15	"
Oenocytes	70—90	70—75	7
Nuclei of oenocytes	30—33	24	"
Fat cells	60	23—35	"
Nuclei of fat cells	20—25	12—15	"
Oenocytes	75—95	70—77	7—8
Nuclei of oenocytes	30—36	21	"
Fat cells	60—75	36—40	"
Excretal cells	42	100	"

Length of middle leg in microns (Length of primordia [anlagen] without thorax forming portion) (Jaeckel ⁷).

Age of larvae in days	Length of middle leg in microns	
	Normal (healthy)	Pathological (American f.b.)
2	90	130—150
3	115—130	130—160
4	180	160—190
5	210	210—230
6	approx. 300	210—240
7	not measurable	265

Length of oesophagus without the Valvula cardiaca (Jaeckel ⁷).

Age of larvae in days	Length of oesophagus in microns	
	Normal	Pathological
2	295	380
3	510	595
4	595—680	765 (in excep-
5	850	770 tional cases
6	1140	785 1690)
7	—	810

Size of ovaries (Jaeckel ⁷).

Age of larvae in days	Size of ovaries in microns	
	Normal	Pathological
2	155 long 40 wide	375 long 69 wide
3	300 long 40 wide	440 long 95 wide
4	355 long 70 wide	460 long 100 wide
5	540 long 110 wide	840 long 120 wide
6	540 long 100 wide	1890 long 391 wide
7	680 long 105 wide	2550 long 400 wide
7		Naturally infected 680—850 long 136 wide
7—8	664 long 100 wide	1360 long 130 wide

Approximate ratio: ovary length to body length (Jaeckel ⁷)

Age of larvae in days	Approximate Ratio: ovary length/body length	
	Normal	Pathological
2	1 : 18	1 : 10
3	1 : 15	1 : 10
4	1 : 17	1 : 10
5	1 : 14	1 : 8
6	1 : 17	1 : 4
7	1 : 20	1 : 3
7—8	1 : 20	1 : 6

The oenocyte number (the values given are only approximate (Jaeckel ⁷)).

Age of larvae in days	Approximate oenocyte number	
	Normal	Pathological
2	19 longitudinal section	25
	8 cross section	—
3	42 longitudinal section	67
	16 cross section	—
5	52 longitudinal section	54
6	36 " "	45
	13 cross section	—
7	37 longitudinal section	62

The results incorporated in the above tables shown that a marked difference in size is frequently noticeable between cells of healthy larvae and those affected with American foul brood.

III. European foul brood

1. The seasonal distribution of European foul brood.

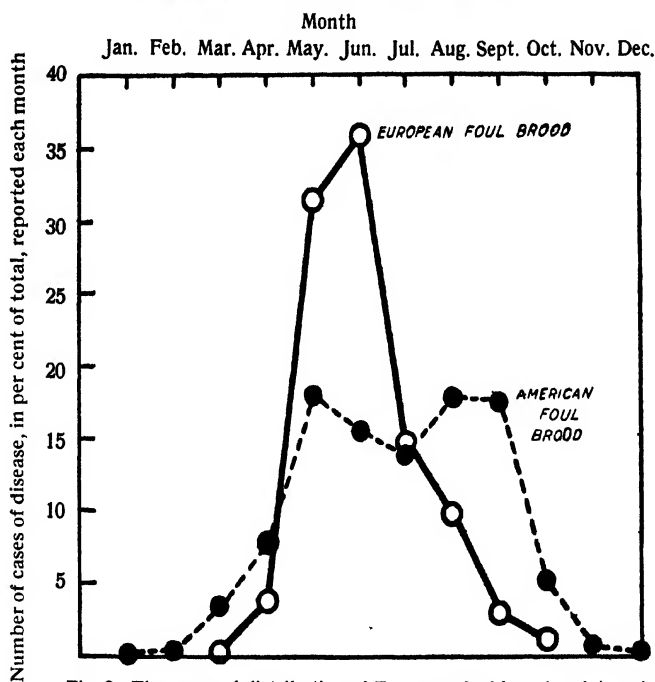


Fig. 2. The seasonal distribution of European foul brood and American foul brood in Switzerland (Morgenthaler ³²).

From this figure it is evident that, while American foul brood can be found at almost any time during the brood-rearing season, European foul brood exhibits a marked seasonal variation in its occurrence. Thus it is scarcely noticeable early in the broodrearing season, reaches a sharp peak in May and June when brood-rearing activities are most pronounced,

and practically disappears toward the end of the season. This fact has been recorded by both Sturtevant³⁵) and Phillips³⁴).

*The distribution of European foul brood by months, including all positively diagnosed samples received from 1906 to 1917 (Phillips³⁴)).

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
No. samples received	3	4	17	33	180	334	240	164	98	17	7	2

*) Data from the bee Culture Laboratory, Washington, U.S.A.

2. The organism which is considered responsible for European foul brood (*Bacillus pluton* White) has not yet been cultivated on laboratory media (White⁵²); Morgenthaler²⁵); Tarr^{43, 45, 48}).

All experiments using *B. pluton*, the results of which have been incorporated in the following tables, have been carried out with suspensions of the organism from the intestines of recently infected larvae.

3. The resistance of *B. pluton* to heat, sunlight and certain disinfectants (White^{50, 52})

The resistance of *B. pluton* to heat when suspended in water (White⁵⁰)).

Temperature °C.	Period of heating in mins.	Results of inoculating healthy colonies of bees
75—80	10	No disease produced
65—70	"	" " "
64—66	"	" " "
64—65	"	" " "
**62—63	"	" " "
**62—63	"	European foul brood produced
**62—63	"	" " " "
61—62	"	" " " "
60	"	" " " "
60	"	" " " "
58—60	"	" " " "
57—60	"	" " " "
55—60	"	" " " "

**) In cases where the same temperature is recorded more than once more than one experiment was performed.

The resistance of *B. pluton* to heat when suspended in honey (White⁵²)).

Temperature °C.	Period of heating in mins.	Results of inoculating healthy colonies of bees
67	10	European foul brood produced
70	"	" " " "
75	"	" " " "
76	"	" " " "
78	"	" " " "
79	"	No disease produced
80	"	" " "
80	"	" " "
81	"	" " "
85	"	" " "
90	"	" " "

The resistance of *B. pluton* to drying at different temperatures (White ⁵²).

Temperature	Period of exposure		Results of inoculating healthy colonies of bees
	Months	Days	
37° C. (Incubator temperature)	0	10	European foul brood produced
	0	17	" " " "
	0	24	" " " "
	1	2	" " " "
	1	7	" " " "
	1	17	" " " "
	2	0	" " " "
	2	11	" " " "
	3	0	" " " "
	9	10	" " " "
	12	2	" " " "
	12	0	No disease produced
	14	18	" " "
	14	20	" " "
	24	0	" " "
Room temperature	1	1	European foul brood produced
	1	21	" " " "
	2	18	" " " "
	2	21	" " " "
	3	0	" " " "
	3	14	" " " "
	9	10	" " " "
	12	6	" " " "
	11	13	No disease produced
	11	15	" " "
	11	18	" " "
	12	2	" " "
	14	10	" " "
	14	18	" " "
	24	0	" " "
	36	0	" " "
Outdoor temperature	0	33	European foul brood produced
	0	47	" " " "
	0	74	" " " "
	9	21	" " " "
	10	18	" " " "
	13	17	" " " "
	12	3	No disease produced
	12	17	" " "
	23	0	" " "
(Refrigerator temperature) About 0° C.	0	26	European foul brood produced
	3	28	" " " "
	6	12	" " " "
	8	0	" " " "
	8	2	" " " "
	9	0	" " " "
	10	7	" " " "
	10	18	" " " "

The resistance of *B. pluton* in a dry film exposed to direct sunlight (White ⁵²).

Period of exposure in hrs.	Results of inoculating healthy colonies of bees
3	European foul brood produced
6	" " " "
7	" " " "
8	" " " "
10	" " " "
14	" " " "
15	" " " "
16	" " " "
*20	" " " "
*20	" " " "
21	" " " "
23	" " " "
27	" " " "
21	No disease produced
23	" " "
24	" " "
*26	" " "
*26	" " "
31	" " "
38	" " "
40	" " "
44	" " "
46	" " "
63	" " "
95	" " "

The resistance of *B. pluton* in aqueous suspensions exposed to direct sunlight (White ⁵²).

Period of exposure in hrs.	Results of inoculating healthy colonies of bees
1	European foul brood produced
* 2	" " " "
* 2	" " " "
* 3	" " " "
* 3	" " " "
* 3	" " " "
* 3	" " " "
4	" " " "
* 5	" " " "
* 5	No disease produced
* 5	" " "
6	" " "
* 7	" " "
* 7	" " "
8	" " "
9	" " "
10	" " "
18	" " "

The resistance of *B. pluton* in honey suspensions exposed to direct sunlight (White ⁵²).

Period of exposure in hrs.	Results of inoculating healthy colonies of bees
1	European foul brood produced
2	" " " "
* 3	" " " "
* 3	No disease produced
* 3	" " "
* 3	" " "
4	" " "
* 5	" " "
* 5	" " "
6	" " "
7	" " "
8	" " "
9	" " "
10	" " "

*) More than one experiment carried out.

The resistance of *B. pluton* to phenol in aqueous suspensions (White

Period of exposure		Phenol concentration %	Results of inoculating healthy colonies of bees
days	hrs.		
0	18	0,5	European foul brood produced
1	0	0,5	" " " "
4	0	0,5	" " " "
8	0	0,5	" " " "
18	0	0,5	No disease produced
0	5	1,0	European foul brood produced
4	0	1,0	No disease produced
0	5	2,0	" " "
0	18	2,0	" " "
1	0	2,0	" " "
4	0	2,0	" " "
4	0	2,0	" " "
9	0	2,0	" " "
0	5½	4,0	" " "

The resistance of *B. pluton* to various chemical compounds when suspended in honey and fed to healthy colonies of bees (White⁵²).

Compound	Concentration	Results of inoculating healthy colonies of bees
<i>β</i> -naphthol	1 : 2000	European foul brood produced
"	1 : 1000	" " " "
"	1 : 500	" " " "
Phenol	1 : 2000	" " " "
"	1 : 1000	" " " "
"	1 : 500	" " " "
Oil of Eucalyptus	1 : 250	" " " "
" " "	1 : 250	" " " "
Formic acid	1 : 1000	" " " "
" "	3 : 1000	" " " "
Salicylic acid	1 : 2000	" " " "
" "	1 : 1000	" " " "
" "	1 : 500	" " " "
Phenyl salicylate	1 : 2000	" " " "
" "	1 : 1000	" " " "
" "	1 : 500	" " " "
Quinine bisulphate	1 : 500	" " " "
" "	1 : 250	" " " "
" "	1 : 100	" " " "

As in the case of American foul brood no protection against European foul brood is afforded by any of the chemical compounds listed in the concentrations given.

4. The occurrence of *B. pluton* in the intestinal tracts of adult bees.

The occurrence of *B. pluton* in the intestinal tracts of nurse bees taken from 7 colonies affected with European foul brood (Sturtevant³⁵)).
(Data obtained from microscopical examinations).

Total number of bees examined	No. containing <i>B. pluton</i>	No. containing <i>B. alvei</i> but doubt- ful <i>B. pluton</i>	No. showing no sign of <i>B. pluton</i>
117	9	11	97

The presence of *B. pluton* in the rectal ampullae of nurse and house cleaning bees taken from colonies affected with European foul brood has also been recorded by Tarr^{43, 45})).

5. The pathogenicity of certain bacteria occurring in larvae affected with European foul brood for the brood of healthy colonies of bees.

European foul brood is a disease in which *B. pluton* is the primary invader of the larvae (the causal organism), but in which numerous other bacteria occur in the affected brood (the so-called „secondary invaders”) (White⁵²); Tarr^{43, 45, 48})). The figures given in the following table show that *B. pluton* is pathogenic for healthy larvae, and that, as with *B. larvae* spores in the case of American foul brood, a fairly large inoculum (mass inoculum) of the cells of this organism is necessary to produce European foul brood in healthy colonies of bees. It is also evident from the results given that none of the secondary invaders can cause European foul brood, and that any question of a filterable virus being implicated in the etiology of the disease can also be discounted.

The results of inoculating healthy colonies of bees with certain of the bacteria commonly occurring in larvae affected with European foul brood (Tarr 44, 45) and unpublished observations).

Organism employed	Source of organism	Size of inoculum (No. of cells)	Method of introducing the inoculum into the healthy colony	Results of inoculation
<i>Bacillus plauton</i>	Stomachs of young larvae recently affected with European foul brood	98×10^6	Fed to the bees in 30 cc of sugar syrup	No disease produced
"	"	490×10^6	" " " " 30 " " "	European foul brood produced
"	"	980×10^6	" " " " 30 " " "	" " " "
"	"	700×10^6	Sprayed over the developing brood in 25 ml of water	" " " "
<i>Streptococcus apis</i> (5a) (so-called)	Pure culture	10.4×10^7	500 individual larvae fed in 0.005 cc	No disease produced
" (5a) "	"	12×10^8	350 " " " 0.0025 "	" " "
<i>Bacillus alvei</i> (spores)	"	5.4×10^7	300 " " " 0.005 "	" " "
" "	"	2.7×10^8	450 " " " 0.0025 "	" " "
<i>Streptococcus apis</i> (so-called) ..	"	1.4×10^{13}	50 cc sprayed over developing brood	" " "
" " ..	"	176×10^{10}	Fed to bees in 200 cc sugar syrup ...	" " "
<i>Bacillus alvei</i> (spores)	"	13.7×10^{11}	" " " 150 " " ...	" " "
<i>Streptococcus apis</i> (so-called) ..	"	315×10^9	25 ml. sprayed over developing brood	" " "
<i>Bacillus para-alvei</i> (48a) (spores).	"	30×10^{10}	15 " " " " "	" " "
<i>Bacillus alvei</i> (spores)	"	22×10^{10}	20 " " " " "	" " "
<i>Bacterium eurydice</i>	"	23×10^{10}	10 " " " " "	" " "
"	"	30×10^{10}	20 " " " " "	" " "
Bacterial-free extracts from affected larvae (to test for virus)			Sprayed or fed to the bees	" " "

IV. Sac brood

The disease „Sac brood” (White ⁵¹) is caused by a filterable virus.

The effect of heat on the virus causing Sac brood when suspended in various media (the virus being extracted from larvae affected with the disease) (White ⁵¹).

Suspending medium	Temperature °C.	Period of exposure	Results of inoculating healthy colonies of bees
Water	50	30	Sac brood produced
„	*55	10	„ „ „
„	*55	20	„ „ „
„	57	15	„ „ „
„	*58	10	„ „ „
„	*58	10	No disease produced
„	59	10	„ „ „
„	60	15	„ „ „
„	61	10	„ „ „
„	65	15	„ „ „
„	70	15	„ „ „
„	75	15	„ „ „
„	80	15	„ „ „
Glycerine	60	10	Sac brood produced
„	65	10	„ „ „
„	70	10	„ „ „
„	71	10	„ „ „
„	73	10	No disease produced
„	75	10	„ „ „
Honey	60	10	Sac brood produced
„	63	10	„ „ „
„	65	10	„ „ „
„	68	10	„ „ „
„	69	10	„ „ „
„	*70	10	„ „ „
„	*70	10	No disease produced
„	*70	10	„ „ „
„	*71	10	„ „ „
„	*71	10	„ „ „
„	*73	10	„ „ „
„	*73	10	„ „ „
„	*75	10	„ „ „
„	*75	10	„ „ „
„	80	10	„ „ „

*) More than one experiment carried out.

The resistance of the virus of Sac brood to drying at room temperature (White ⁵¹).

Length of exposure		Results of inoculating healthy colonies of bees
Months	Days	
0	3	Sac brood produced
0	7	" " "
0	13	" " "
0	16	" " "
0	18	" " "
0	20	" " "
0	22	No disease produced
0	26	" " "
0	28	" " "
0	28	" " "
0	35	" " "
0	45	" " "
7	12	" " "
7	21	" " "

The resistance of the virus of Sac brood in a dry film exposed to direct sunlight (White ⁵¹).

Period of exposure hrs.	Results of inoculating healthy colonies of bees
2	Sac brood produced
2½	" " "
3	" " "
4	" " "
5	" " "
6	" " "
6	" " "
4	No disease produced
5	" " "
7	" " "
9	" " "
12	" " "
13	" " "
18	" " "
21	" " "

The resistance of the virus of Sac brood to sunlight when suspended in water (White ⁵¹).

Period of exposure in hrs.	Results of inoculating healthy colonies of bees
1	Sac brood produced
* 2	" " "
* 2	" " "
* 2	" " "
3	" " "
* 4	" " "
* 4	" " "
* 5	" " "
* 4	No disease produced
* 5	" " "
* 5	" " "
* 5	" " "
6	" " "
7	" " "
8	" " "
10	" " "
12	" " "
*13	" " "
*13	" " "

The resistance of the virus of Sac brood to sunlight when suspended in honey (White ⁵¹).

Period of exposure in hrs.	Results of inoculating healthy colonies of bees
1	Sac brood produced
2	" " "
* 4	" " "
* 4	" " "
* 4	" " "
5	" " "
* 5	No disease produced
* 5	" " "
* 5	" " "
6	" " "
7	" " "
8	" " "
10	" " "
12	" " "
13	" " "
18	" " "

*) More than one experiment carried out.

The survival of the virus of Sac brood in honey (White ⁵¹).

Period of exposure		Results of inoculating healthy colonies of bees
Months	Days	
0	20	Sac brood produced
0	23	" " "
0	30	" " "
0	24	No disease produced
0	29	" " "
0	33	" " "
0	35	" " "
0	36	" " "
0	49	" " "
0	70	" " "
7	10	" " "
7	20	" " "
8	2	" " "
8	21	" " "
12	1	" " "

The resistance of the virus of Sac brood to phenol (White ⁵¹).

Period of exposure		Phenol Concentration %	Results of inoculating healthy colonies of bees
hrs.	Days		
0	1	0,5	Sac brood produced
0	16	0,5	" " "
0	24	0,5	" " "
0	38	0,5	No disease produced
0	*50	0,5	" " "
0	*50	0,5	" " "
0	238	0,5	" " "
0	1	1,0	Sac brood produced
0	16	1,0	" " "
0	25	1,0	" " "
0	38	1,0	No disease produced
0	*50	1,0	" " "
0	*50	1,0	" " "
0	251	1,0	" " "
0	1	2,0	Sac brood produced
0	16	2,0	" " "
0	25	2,0	" " "
0	38	2,0	No disease produced
0	42	2,0	" " "
0	50	2,0	" " "
3	0	4,0	Sac brood produced
7	0	4,0	" " "
0	25	4,0	No disease produced
0	50	4,0	" " "

*) More than one experiment carried out.

V. Chalk brood.

1. Seasonal occurrence, sex of larvae affected, and age at which the larvae are affected.

The seasonal occurrence of Chalk brood in Switzerland for the period 1917—1933 (Maurizio ¹²).

(For a description of so-called „Primary” and „Secondary” Chalk brood, see Maurizio ¹²).

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
No. of cases	0	0	0	1	14 (1) *	21 (6) *	8	4 (2) *	3	1	0	0

*) Secondary Chalk brood.

The sex of larvae affected with Chalk brood (1917—1933)
(Maurizio ¹²).

Description of larvae affected	No. of cases of Chalk brood	
	Primary	Secondary
Normal drone brood ♂	11	0
Drone brood from a drone laying queen (Buckelbrut) ♂	6	1
Worker brood ♀	14	8
Worker and drone brood in the same hive	9	0
Worker brood and „Buckelbrut” in the same hive	1	0
No available data	11	0

These results show that Chalk brood, as European foul brood, reaches a sharp peak during the active part of the brood-rearing season (May and June). The evidence presented also indicates that drone larvae and worker larvae are almost equally susceptible to Chalk brood.

The age at which larvae are affected with Chalk brood (Maurizio ¹²).

Colony of bees examined	Total no. of cells examined	Cells unsealed by the bees during the first 6 days after sealing			Unsealed cells found from 7—13 (worker brood) and 7—19 (Drone brood) days after sealing. (Largely due to the emergence of young bees)				
		No. of cells unsealed	No of larvae affected with Chalk brood	No of empty cells*	No. of cells unsealed	No. of larvae affected with Chalk brood	No. of larvae hatched	No. of empty cells	
Control (healthy).	136	2 (1,46 %)	0	2 (100 %)	134 (98,5 %)	0	132 (98,5 %)	2 (1,5 %)	
Affected with Chalk brood (only worker brood exam.)	1686	244 (14,5 %)	155 (63,3 %)	89 (36,6 %)	1142 (85,4 %)	0	1405 (97,9 %)	37 (2,1 %)	
Affected with Chalk brood (only worker brood exam.)	133	23 (17,4 %)	12 (52,1 %)	11 (47,9 %)	110 (82,6 %)	0	93 (84,2 %)	17 (15,8 %)	
Affected with Chalk brood (only drone larvae exam.) („Buckelbrut“).	333	152 (45,6 %)	2 (2,6 %)	148 (97,4 %)	181 (54,3 %)	0	135 (74,5 %)	46 (25,5 %)	

*) In the colonies affected with Chalk brood many of these empty cells had probably contained „Chalk brood larvae“ which the bees had removed.

A comparison of the percentage of unsealed cells found in healthy colonies of bees and in colonies affected with Chalk brood (Maurizio ¹²).

Colony of bees examined	Total no. of cells exam.	*No. of brood cells unsealed by the bees of the colony and by emerging bees (in % of the total number examined) in the following no. of days after sealing:																			
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Control (healthy).....	136			0,73		0,73					0,73	0,73	80,2	9,5							
Affected with Chalk brood (only worker brood exam.)	1686		0,23	4,43	5,7	2,68	1,17	0,29	0,23	0,23	0,95	5,7	22,8	48,2	6,5	0,83					
Affected with Chalk brood (only worker brood exam.)	133		1,5	1,5	4,65	5,2	0,75	3,8	5,2	1,5		0,75	9,8	40,6	18,7	6,05					
Affected with Chalk brood (Buckelbrut) (only drone larvae examined)	333	1,5	12,0	16,2	9,6	3,6	1,8	0,9	2,2	2,2	2,5	1,8	0,9	1,8	8,72	22,29	9,1	0,33	0,9	0,9	0,66

*) In this table the number of unsealed cells found up to the 9 th. day after sealing is almost entirely due to removal of larvae affected with Chalk brood by the bees. The empty cells found after the 9 th. day are largely due to the emergence of healthy adult bees.

The results given in the above tables indicate that larvae are only attacked by Chalk brood during the first six days after the cells containing them have been sealed by the bees, and that most of the infected larvae are removed by the bees before the ninth day following sealing.

2. Distribution of sexes, varieties, size of fruiting bodies, and influence of temperature on *Pericystis apis*, the Chalk brood fungus.

The occurrence of the two sexes of *P. apis* in samples studied in Switzerland from 1917—1933 (Maurizio¹²).

Description of sample of comb.	Number of cases	
	Primary Chalk brood	Secondary Chalk brood
Combs containing both sexes of the fungus and fruiting bodies	26	6
Combs containing both sexes of the fungus in different larvae	2	0
Combs containing only the mycelium of the positive (♀) sex	8	2
Combs containing only the mycelium of the negative (♂) sex	2	0
Without information	14	1

Two varieties (forms) of *P. apis* have been distinguished, a large and a small fruiting body type (Maurizio¹³), and the following tables record the rate of growth, the size of fruiting bodies and the influence of temperature on fruiting body formation in both these forms.

The average daily growth of both forms of *P. apis* in mm. (Maurizio¹³).

	Growth in mm at the following temperatures: (°C.)							
	0	11	15	20	25	30	37	42
	0	11	15	20	25	30	37	42
Small fruiting form..... + (♀)	0	2,26	3,86	7,12	10,9	12,52	7,52	0
" " " — (♂)	0	2,4	4,75	7,12	11,45	14,22	10,4	0
" " " (♀ + ♂)	0	2,32	4,2	7,12	11,7	13,27	8,68	0
Large " " (♂ ?)	0	3,2	4,9	8,94	11,3	11,96	1,3	0
" " " (♀ ?)	0	3,15	4,8	9,4	10,25	11,00	2,45	0
" " " (♂ + ♀)	0	3,17	4,86	9,14	10,88	11,56	1,76	0

The influence of temperature on fruiting body formation by both forms of *P. apis* expressed as the percentage of the strains examined which formed such bodies. (Maurizio¹³).

	Temperature in °C.								No. of „crossings” made *
	0	11	15	20	25	30	37	42	
Large fruiting form ...	0	55	74	84,2	61,4	2,2	0	0	32
Small " " ...	0	7	62,2	67,8	86	100	56,6	0	19

*) 16 small and 24 large fruiting strains were studied in various „crossings” of ♀ and ♂

The size of the fruiting bodies of both forms of *P. apis* (Maurizio 13).

Origin of fruiting body	Number of measurements made	Numbers of fruiting bodies found and their diameters in microns												Avg. size with avg. error μ	Standard deviation with avg. error μ	Variation coefficient
		20	40	60	80	100	120	140	160	180	200	220	240	260	280	
		1	79	235	138	34	12	1								
Small fruiting form:																
From affected larvae	500	—	94	230	157	18	1	—								25,85
" pure cultures	500	—	94	230	157	18	1	—								22,71
Totals	1000	1	173	465	295	52	13	1								24,68
Large fruiting form																
From affected larvae	500	—	1	18	65	77	61	79	60	69	35	24	6	5	—	32,75
" pure cultures	2000	—	3	87	240	417	470	328	227	99	71	27	15	12	4	29,72
Totals	2500	—	4	105	305	494	531	407	287	168	106	51	21	17	4	31,19

The influence of temperature on the size of the fruiting bodies of both forms of *P. apis* (Maurizio 13).
 (100 fruiting bodies of the small form, and 250 of the large form were measured; they were obtained from pure cultures).

Temperature °C.	Small fruiting form				Large fruiting form			
	Average size in μ , with average error	Standard deviation with average error	Variation coefficient		Average size in μ , with average error	Standard deviation with average error μ	Variation coefficient	
11					146,28 \pm 2,77	43,88 \pm 1,95	29,99	
15	56,6 \pm 1,71	17,16 \pm 1,21	30,32		173,08 \pm 3,23	51,28 \pm 2,29	29,63	
20	59,48 \pm 1,99	19,9 \pm 1,33	33,46		161,28 \pm 3,22	51,— \pm 2,28	31,62	
25	62,32 \pm 1,81	18,1 \pm 1,27	29,05		105,64 \pm 2,05	32,58 \pm 1,4	30,84	
30	66,76 \pm 2,38	23,83 \pm 1,61	35,69		78,72 \pm 2,42	19,66 \pm 1,71	24,97	
37	64,24 \pm 1,62	16,24 \pm 1,14	25,28					

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O. MORGENTHALER, Bienen-Krankheiten	} folgen. will follow.
D. MORLAND, Healthy Bee	
	} suivront.

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STUDIES ON EUROPEAN FOUL BROOD OF BEES

II. THE PRODUCTION OF THE DISEASE
EXPERIMENTALLY

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(With Plates XXII-XXIV)

THE etiology of so-called European foul brood of bees has long remained in doubt in spite of considerable investigation. Since numerous summaries of the pertinent literature are available (3, 4, 5, 10, 15, 16) an exhaustive résumé of the historical aspects of the problem is unnecessary, and in this paper, therefore, the results of a number of experiments dealing with the production of the disease are discussed in detail with reference to their value in establishing its cause.

EXPERIMENTAL

Media for cultivation of the bacteria

Hartley's tryptic digest (beef) broth was employed, and agar (1.5 per cent.) and gelatin (12 per cent.) media were prepared from this. Sturtevant's egg agar (egg-yeast-carrot-peptone) was used (14). "Brood-filtrate agar" was prepared by adding 1 ml. of a 1 : 10 solution of brood filtrate (21) to each 5 ml. of beef-digest agar. Nitrate broth was made by adding 0.1 per cent. pure potassium nitrate to the beef-digest broth. The following medium was employed to study hydrogen sulphide formation: Lemco beef extract 1 per cent., peptone (B.D.H.) 2 per cent., lead acetate 0.02 per cent., cystine 0.02 per cent. and agar 1.5 per cent. The cystine was sterilized separately in water and was added as a suspension to the agar medium immediately prior to sloping. All the above media were adjusted to pH 7.0 and were sterilized by autoclaving. Fresh skim milk was employed and was sterilized (with or without litmus indicator) by intermittent steaming. A basic medium consisting of Witte peptone 2 per cent., sodium chloride 0.5 per cent., with 4 ml. of an 0.4 per cent. solution of brom cresol purple per litre was employed to detect the formation of acid from various carbon compounds by the bacteria studied. With the exception of raffinose and fructose, which were sterilized

by Seitz filtration, all the fermentable compounds investigated were sterilized separately by autoclaving in 4 per cent. aqueous solution, and were added to the basic medium to make a final concentration of 1 per cent. Indole and nitrite were tested for as previously described (16).

Source of infective material and bacterial cultures

All the naturally infected material employed in this investigation was obtained from a colony of hybrid bees in which "European foul brood" had been initiated by the insertion of an infected brood comb in the autumn of 1934. The disease appeared in the spring in several of the larvae which were being reared in the original infected comb, and rapid spread was facilitated by removing a large proportion of the sealed brood combs together with the adhering bees from the colony early in the brood-rearing season, and replacing these with drawn comb or foundation, thus causing extensive weakening of the colony. *Bacillus alvei*, *Streptococcus apis* and a small rod-shaped non-spore-forming bacterium (*Bacterium eurydice?* (20)) were constantly encountered in the infected larvae examined. Larvae were normally attacked when they were about 4 days old, but dark brown, slimy, evil-smelling larvae full of *B. alvei* spores and vegetative cells were fairly frequently encountered in capped cells. In most respects this case of disease conformed with the "European foul brood" described by White as typical (20).

Pure cultures of *B. alvei* were isolated from naturally or experimentally infected larvae by grinding them up in sterile water and "plating" the resulting suspension on brood-filtrate agar after making suitable dilutions. The plates were "dried" at 35° C. for a short time after pouring in order to obviate the "spreading" of surface colonies. Stock spore-containing cultures of the various isolated strains of *B. alvei* were prepared by inoculating egg-agar slopes directly from the isolated colonies which developed on the plates and incubating them for 5-7 days at 35° C. The resulting cultures were sealed with paraffin wax and were stored at room temperature until they could be identified.

Pure cultures of *S. apis* were obtained in much the same manner as those of *B. alvei*, except that recently infected larvae were found to be most suitable for the isolation of this species, and drying of the plates was unnecessary. Stock cultures of the isolated strains were prepared from primary colonies by inoculating brood-filtrate agar slopes, incubating them for 12-16 hours at 35° C., and sealing the resulting culture. Culture 1 (*B. alvei*) and culture 2 (*S. apis*) have been employed in all the infection experiments, and normally all cultures have been incubated at 35° C.

*Identification of isolated cultures of Bacillus alvei
and Streptococcus apis*

Streptococcus apis strains.

Although Borchert⁽⁵⁾ has described *S. apis* in some detail it has been thought advisable to discuss the characteristics of this organism here in view of the fact that certain important differences have been noted among the strains examined. All the media employed in the identification of the various strains were inoculated from 12-24-hour-old brood-filtrate agar cultures which had been inoculated directly from the stock cultures. The number of each culture refers to a given experiment (*vide infra*).

Morphology. On agar media, in broth and in milk, the predominating form is that of a lanceolate-shaped *Diplococcus* (Pl. XXII, figs. 1-4); very occasionally short chains are formed (Pl. XXII, fig. 2). The size of the cells is extremely variable under certain conditions (Pl. XXII, fig. 5). The organism is non-motile, and so far no capsules have been noticed on any of the media. The organism is Gram positive when very young cultures are examined, but the cells rapidly lose their power to retain this stain, and in most cultures an abundance of Gram-negative cells can be seen. A comparison of the cells of this organism with those it forms in larvae sick or dead of "European foul brood" can be obtained from a study of Pl. XXIII, figs. 7, 8, 11 and 12, and Pl. XXIV, figs. 15 and 16.

Cultural and biochemical characteristics. On beef-digest agar the growth is very rapid: it is uniform with regular margin, opaque and rather white, shiny, slightly raised, soft, moist and non-adherent. The addition of brood filtrate accelerates growth and makes it more abundant. Surface colonies on beef-digest agar are circular with regular margin, slightly raised and about 1-2 mm. in diameter. Subsurface colonies are lanceolate shaped. In beef-digest broth growth is followed by uniform clouding of the medium; eventually a heavy precipitate settles, the medium becoming fairly clear. Considerable variation has been found with respect to growth and reaction toward milk and gelatin media. Thus cultures 1, 6, 9 and 11 when grown in litmus milk rapidly reduce the indicator and coagulate the casein, almost completely peptonizing it within a week. After 1 week only a small amount of red undigested casein and a clear almost yellow whey remains. The milk is quite acid. In beef-digest gelatin stab cultures these strains grow quite well at 22° C., and within 48 hours there is a marked saccate liquefaction of the medium. At 35° C. there is rapid and complete liquefaction of gelatin.

On the other hand, cultures 3, 4, 5, 7, 8 and 10 neither hydrolyse casein nor gelatin. Thus growth of these strains in litmus milk is followed by a mere transient reduction of the indicator which soon becomes re-oxidized, leaving a faintly acid uncoagulated medium. In gelatin stab cultures at 22° C. the growth is uniform and filiform and no liquefaction occurs. Similarly at 35° C. no liquefaction of the gelatin results even after 3 weeks. None of the strains studied reduce nitrate to nitrite, and none form hydrogen sulphide or indole in old milk cultures or in broth. All strains investigated exhibit the same fermentative power. Thus acid is formed from dextrin, sucrose, lactose, maltose, glucose, fructose, galactose, mannose, mannitol, glycerol and salicin by all the cultures, and none of them produce acid from starch, inulin, raffinose, arabinose, xylose, inositol, adonitol and erythritol under the experimental conditions. Borchert⁽⁵⁾ found that the strains of *S. apis* investigated by him produced acid in small amounts from raffinose, but it must be remembered that fermentation reactions of a given species are liable to significant variations depending on the nitrogen substrate employed in its cultivation^(11, 12). It is of interest that the species hitherto known as *S. apis* is apparently divisible into subspecies depending on the power of hydrolysing casein and gelatin. In all other respects the various strains studied appear to be identical.

Bacillus alvei strains.

All the strains of *B. alvei* isolated (cultures 2, 12, 13, 14, 15, 16, 17 and 18) exhibited the same general cultural, morphological and biochemical characteristics as has been described for certain other cultures⁽¹⁶⁾. The fermentation reactions of all the cultures isolated was found to be identical with those given by cultures 3 and 4 described previously⁽¹⁶⁾, with the single exception that raffinose was not fermented.

Preparation of material for infection experiments

Suspensions of the spores of *B. alvei* (strain 2) and vegetative cells of *S. apis* (culture 1) were prepared as follows. Suspensions of these organisms were prepared in sterile water directly from the original stock cultures in order to avoid too many transfers away from the natural host and the consequent danger of loss of virulence. From these egg agar, in the case of *B. alvei*, and brood-filtrate agar, in the case of *S. apis*, were inoculated employing large amounts of these media in Petri dishes or culture bottles. The resulting cultures were incubated 5-7 days in the case of *B. alvei*, and 12-16 hours in the case of *S. apis*. The spores or

vegetative cells were then washed carefully from the medium employing sterile water, centrifuged in order to concentrate them, and finally suspended in water to give a relatively dense suspension. The approximate number of spores or vegetative cells present in such suspensions was estimated by making dilutions and counting the number of bacteria by means of a Thoma haemocytometer slide.

Filtrates of infected brood were prepared by grinding up larvae sick or dead of "European foul brood" in water, adjusting the pH of the resulting suspension to 7.2 or 7.4, allowing the mass to stand approximately half an hour at 35° C., centrifuging at a low speed to remove the larger particles of cell debris, and finally filtering the supernatant liquid through Pasteur-Chamberland L 2 or L 3 filters, employing suction. Filtrates obtained in this manner were normally opalescent, and in each instance their sterility was verified by plating 2 ml., employing brood-filtrate agar as substrate and incubating the plates 2 days at 35° C. and 5-7 days at room temperature.

Preparation of experimental nuclei employed in infection experiments

It is naturally impossible to obtain any absolutely standard nucleus or colony of bees for experimental work, since there are so many uncontrollable variables, such as the ratio of "nurse" and "house cleaning" bees to brood at different stages in the brood-rearing season, the relative amount of sealed and unsealed brood present, the effect of honey flow, etc. In addition the race of bee undoubtedly influences the susceptibility of a given colony of bees to "European foul brood", Italian races being particularly resistant in this respect⁽¹³⁾. In all the experiments recorded in this paper hybrid bees have been used owing to force of circumstances, and it seems highly probable that the resistance toward this disease displayed by certain nuclei depended to some extent at least on the amount of Italian strain in the bees. Since no standard of strength of an experimental nucleus is available it has not been possible to give an exact statement regarding the strength of a given nucleus in bees, and only in cases where the nucleus was obviously strong or weak in bees has a note to this effect been made. All experiments recorded in this paper have been carried out in single-walled nucleus hives capable of taking five British Standard brood frames. The entrance consisted of a 2 by $\frac{1}{2}$ in. slot cut in the front of the hive, and the frames were covered by a piece of "Ten Test" board which had a $1\frac{1}{2}$ in. diameter hole bored in the centre covered with $\frac{1}{8}$ in. wire gauze for a "feed hole". Each hive was equipped with a deep roof capable of covering the feeder used. Feeding

was carried out using 1 or $\frac{1}{2}$ lb. Ministry of Agriculture "squat" honey jars with five $\frac{1}{8}$ in. diameter holes bored in the lids. These were filled with the syrup employed and were inverted over the feed hole. After 15 August all the nuclei were fed with 50 per cent. sucrose syrup in order to stimulate brood rearing.

The nuclei were made up by taking three or four brood combs containing chiefly sealed brood and stores (honey and pollen) together with the adhering bees from healthy stocks, and placing these in nucleus hives. The bees of each nucleus were then permitted to raise their own queen. Owing to unfavourable weather conditions none of the experimental nuclei could be prepared before 23 May, and as a result of continued unfavourable conditions the mating of the queens was delayed and hardly any experiment was commenced prior to the last week in June. However, this resulted in considerable weakening of some of the nuclei with respect to the number of bees present, and consequently they were probably more susceptible to "European foul brood" (*vide infra*). As the season progressed the nuclei were given additional frames containing brood comb or foundation in order that there might be room for expansion of the brood nest.

Method of inoculation of experimental nuclei

Two general methods of infecting nuclei have been attempted: namely, indirect inoculation by feeding the bees the infective material, and direct inoculation by feeding young larvae directly. In the indirect method the infectious material was fed, usually mixed with 30 per cent. sterile sucrose syrup containing 10 parts of honey for every 100 parts of syrup, to the bees of a healthy nucleus by means of a feeder. In the direct method of inoculation the following technique was employed.

Brood combs containing eggs and coiled larvae up to the age of approximately 4 days were obtained from healthy colonies of bees. Experience proved that the best way to obtain combs with an abundance of young larvae was to place a frame containing brood foundation in a vigorous healthy stock and permit the bees to draw out cells and to rear the larvae in them. In this manner brood combs containing an abundance of coiled larvae of a relatively uniform age were readily obtained at any time during the active part of the brood-rearing season. For most experiments, especially those with "starved larvae", larvae about 3-4 days old were inoculated, but occasionally infective material was introduced to the base of cells containing eggs, or into the brood-food mass surrounding very young larvae. The actual inoculation was made

by feeding individual larvae either 0.0025 or 0.005 ml. of a suspension of bacteria or infective material by means of an Agla micrometer syringe, the minute drop of liquid being placed near the mouth-parts of the larva with the needle. In certain of the experiments the brood combs containing the inoculated larvae were placed in a healthy nucleus immediately subsequent to inoculation, while in others the larvae were starved in the comb, in an incubator in which the humidity of the air was retained at a fairly high level, for 4 days at 35° C. This starvation technique has been found particularly useful, for the resistance of the larvae is apparently weakened under such conditions, and multiplication of the bacteria in the host is thereby greatly facilitated. In one experiment it is shown that normal coiled larvae are practically sterile bacteriologically. Moreover, it has been observed that, although a very large number of artificially inoculated larvae succumb to infection after starvation, a certain percentage apparently destroy the bacteria, and, at least as far as microscopical examinations are concerned, appear to be sterile bacteriologically after the 4 days. As subsequent experiments will show, inoculated unstarved larvae which are attended to by the nurse bees have a relatively high resistance toward the bacteria. It would seem that larvae possess some potent bactericidal mechanism: perhaps this is merely a function of their digestive system.

Method of examination of inoculated nuclei and of recording experiments

Inspections of the brood of inoculated nuclei were made fairly frequently, care being taken to avoid any undue disturbance of the bees. Where necessary the bees were smoked or shaken from the combs in order to facilitate inspection of the larvae. All larvae which appeared to be diseased, unless there were a very large number, were removed from the cells with sterile forceps, placed in Petri dishes, and taken to the laboratory for examination. In cases in which a very large number of affected larvae were present only a small fairly representative number were examined. Though ordinary microscopical examination of nigrosine or Gram-stained smears from the tissues of affected larvae can usually be relied upon as a satisfactory confirmation that "European foul brood" is present in a nucleus, diagnosis was made more certain by the isolation and identification of one pure culture of *B. alvei* and *S. apis*. In the record of the experiments the term "*S. apis* cells" indicates that lanceolate-shaped cocci of the type shown in Pl. XXIII, figs. 7, 8, 11 and 12, were present in the tissues of affected larvae. Similarly the term "*B. alvei* cells and spores" indicates that vegetative cells or spores

of this organism as shown in Pl. XXIII, figs. 11 and 12, and Pl. XXIV, figs. 14 and 15, were present in affected larval tissues. The "small rod-shaped bacterium" is probably synonymous with White's *Bacterium eurydice* (20). In each experiment the strength of the nucleus employed in stores (honey and pollen) and brood (eggs, young larvae and sealed brood) is recorded; thus the record "stores; eggs and young larvae; young larvae; sealed brood", indicates a nucleus with four brood combs containing the brood or stores as indicated in the combs. The terms "strong" and "weak" with respect to the relative number of bees present in a given nucleus have only been employed where it is very evident that such a condition exists. The date of inoculation has been recorded for each experiment in view of the variation in the ratio of "nurse" and "house cleaning" bees to young larvae at different periods in the brood-rearing season, and the probable influence of this ratio on the susceptibility of a given nucleus to "European foul brood". The data obtained in each experiment has been arranged according to the following plan: (1) Nucleus, (2) Inoculum, (3) Date of inoculation, (4) Results.

RECORD OF EXPERIMENTS

Exp. 1.

Ten larvae approximately 3-4 days old (average weight calculated from twenty larvae was 42 mg.) were carefully removed from their cells employing sterile forceps and were ground up in 10 ml. of sterile water observing aseptic precautions. 0.1 and 1 ml. portions of the resulting suspensions were plated, using 10 ml. of beef-digest agar in each instance. After 48 hours at 35° C. only two colonies developed on the plate which had received 1 ml. of suspension. This result would seem to indicate that normal healthy larvae are almost bacteriologically sterile, but further investigation is required in confirmation of the experiment.

- 1: *Inoculation of experimental nuclei by placing in them brood combs containing naturally or artificially infected larvae, or other form of infective material*

Exp. 2.

Nucleus. Stores; eggs and young larvae; infected comb; sealed brood; sealed brood and stores. Weak in bees.

Inoculum. Brood comb containing approximately eighty-four larvae sick or dead of "European foul brood".

Date of inoculation. 9 July.

Results. 3 days: Most of the infected larvae had been removed from the introduced comb and eggs had been laid in the empty cells. 7 days: No diseased larvae in the introduced comb; three sick or dead larvae in each of the two adjacent combs. Two of these were full of *S. apis* cells and the remaining four showed *S. apis* and *B. alvei* vegetative cells. 10 days: Twenty affected larvae in the introduced comb, fifteen in the remaining combs. Six of these on microscopic examination showed *S. apis* and *B. alvei* cells and small rod-shaped bacteria. 13 days: Fifty affected larvae in the

introduced comb. Ten of these were examined and showed *S. apis*, small rod-shaped bacteria, and in the case of old slimy decaying larvae sporulating cells of *B. alvei* in apparently pure culture. 21 days: Destroyed. (Queen retained with twelve workers for Exp. 31.) Fifty-seven sick or dead larvae were counted in one brood adjacent to the introduced comb. Ten representative affected larvae were examined microscopically: three recently affected larvae showed *S. apis* cells and a varying proportion of small rod-shaped bacteria; seven slimy, evil-smelling dead larvae, some from sealed cells, showed *B. alvei* vegetative cells and spores in apparently pure culture. *S. apis* (culture 8) and *B. alvei* (culture 12) were isolated in pure culture from affected larvae.

In this experiment very rapid spread of the disease was effected by inserting a comb containing a relatively large number of affected larvae in a healthy nucleus which was rather weak in bees.

Exp. 3.

Nucleus. Stores; eggs and young larvae; inoculated comb of eggs and young larvae; young larvae and sealed brood; eggs and stores.

Inoculum. Ten larvae sick or dead of "European foul brood" were ground up finely in 5 ml. of sterile brood filtrate. Fifty eggs and young larvae up to the age of about 4 days were fed 0.005 ml. of this suspension, and the comb containing them was placed immediately in the nucleus.

Date of inoculation. 19 June.

Results. 2 days: No affected larvae seen, though a few larvae had been removed from their cells. Subsequent careful examinations of the brood 4, 8, 14, 15, 17, 19, 22, 29 and 31 days after inoculation revealed no infection.

The failure to initiate infection with naturally infected material makes this experiment rather interesting. The bees and the queen of the nucleus used were yellow and probably possessed a relatively large proportion of Italian blood, and the failure may well have been due to the strong cleaning powers of the bees.

Exp. 4.

Nucleus. Stores; young larvae (inoculated comb); young larvae and stores.

Inoculum. Fifty larvae sick or dead of "European foul brood" were finely ground in 10 ml. of sterile water. 2000 2-3-day-old larvae on one side of a brood comb were each fed 0.005 ml. of this suspension, and the comb containing them was placed in the nucleus immediately.

Date of inoculation. 2 July.

Results. 2 days: No sign of diseased larvae. A large number of the inoculated larvae had been removed by the bees and eggs laid in the empty cells. 3 days: No sign of diseased larvae. Approximately half the inoculated larvae had been removed by the bees, the remainder were being reared in the normal manner and many were sealed. No sick or dead larvae were noticed during inspection of the brood 5, 8, 10, 12 and 17 days after inoculation. 28 days: Four sick larvae. All of them had an abundance of *S. apis* cells in their tissues, and two of them *B. alvei* cells as well. 41 days: Three sick larvae full of *S. apis* cells were recovered from one comb. *S. apis* (culture 9) was isolated in pure culture from one of these. 46 days: Seven affected larvae were recovered. All of these showed *S. apis* cells and small rod-shaped bacteria, and several of them *B. alvei* cells as well. *B. alvei* (culture 16) was isolated from the remains of one dead larva. 55 days: Four sick larvae full of *S. apis* cells, small rod-

shaped bacteria and *B. alvei* cells. 60 and 65 days: The bees were breeding very slowly and no infected larvae were seen.

In this experiment therefore the disease was merely transient and never assumed serious proportions.

Exp. 5.

Nucleus. Stores; young larvae (inoculated comb); young larvae and sealed brood.

Inoculum. 500 eggs and young larvae 1-4 days old were each given 0.005 ml. of a suspension of *S. apis* cells, each receiving approximately 10.4×10^7 cells of this organism. The comb was then placed in the nucleus immediately after inoculation.

Results. 2 days: Very few of the larvae had been removed by the bees, and there was no sign of infection. 3 days: A large number of the inoculated larvae had been sealed over by the bees. Subsequent examinations of the brood 6, 8, 10, 13, 18, 20 and 21 days after inoculation revealed no infected larvae.

Exp. 6.

Nucleus. Stores; eggs and sealed brood; eggs and young larvae (inoculated comb).

Inoculum. 350 eggs and young larvae 1-4 days old were each given 0.0025 ml. of a very dense suspension of *S. apis* cells, each larva receiving approximately 12×10^8 organisms. The comb containing the inoculated larvae was then placed immediately in the experimental nucleus.

Date of inoculation. 5 July.

Results. 2 days: A few of the eggs and young larvae had been removed by the bees, no infected larvae were seen. Subsequent inspection of the brood at intervals of 4, 5, 8, 10, 13, 17, 20 and 24 days after inoculation revealed no sick or dead larvae.

Exps. 5 and 6 indicate that the individual larvae have a high resistance toward *S. apis* vegetative cells in very large numbers.

Exp. 7.

Nucleus. Sealed brood; eggs and young larvae (inoculated comb); sealed brood; stores.

Inoculum. 300 eggs and young larvae from 1-4 days old were each given 0.005 ml. of a suspension of *B. alvei* spores, each larva receiving approximately 5.4×10^7 spores. The comb was immediately suspended in the nucleus.

Date of inoculation. 13 June.

Results. 4 days: An almost perfect patch of sealed brood present at the site of inoculation, indicating that very few larvae, if any, had been removed by the bees. Careful inspection of the brood at intervals of 9, 14, 21, 27, 32, 37, 42, 48, 55, 58 and 73 days after inoculation revealed no infected larvae.

Exp. 8.

Nucleus. Young larvae and eggs (inoculated comb); sealed brood; young larvae and eggs. Stores present in the brood combs.

Inoculum. 450 young larvae up to 4 days of age and some eggs were given 0.0025 ml. of a very dense suspension of *B. alvei* spores, each receiving approximately 2.7×10^8 spores. The comb containing these was immediately inserted in the nucleus.

Date of inoculation. 5 July.

Results. 2 days: A few larvae had been removed from the cells in the area inoculated but there was no sign of any infected larvae. No infected larvae were seen at sub-

sequent examinations of the brood 4, 5, 10, 14, 20, 25, 28, 33 and 34 days after inoculation.

These two experiments indicate that young larvae when reared normally by nurse bees have a high resistance toward *B. alvei* spores in very large numbers.

Exp. 9.

Nucleus. Stores; eggs and young larvae; eggs and young larvae (inoculated comb); sealed brood.

Inoculum. Twenty "European foul brood" larvae in all stages of disease were triturated with 20 ml. of sterile water, and from the resulting mass a Pasteur-Chamberland L 3 filtrate was obtained as previously described. A portion of this filtrate was mixed with an equal amount of a suspension containing 3.5×10^{10} *B. alvei* spores per ml. Each of 100 larvae 2-4 days old on one side of a brood comb received 0.005 ml. of this suspension, each larva therefore receiving approximately 0.0025 ml. of the filtrate and 8.7×10^7 spores of *B. alvei*. The comb containing the inoculated larvae was placed in the nucleus immediately subsequent to inoculation.

Date of inoculation. 21 June.

Results. 2 days: A few of the larvae in the inoculated area had been removed by the bees. Subsequent inspection of the brood 6, 11, 14, 19, 24, 28, 32, 35 and 41 days after inoculation revealed no infected larvae.

The experiment suggests that a filterable virus is not implicated in this disease.

Exp. 10.

Nucleus. Stores; sealed brood; eggs and young larvae; young larvae (inoculated comb); stores. Weak in bees.

Inoculum. Each of 800, 2-3-day-old larvae were fed 0.0025 ml. of a suspension of *S. apis* cells, individual larvae receiving approximately 5.2×10^7 organisms. The comb containing them was then incubated in an incubator, in which the humidity of the air was maintained at a high level, for 4 days at 35° C. At the conclusion of this time a large proportion of the larvae had become yellow to brown in colour and pappy in consistency and were full of *S. apis* cells. (The appearance of *S. apis* cells in starved larvae is shown in Pl. XXII, fig. 6 and Pl. XXIII, figs. 9 and 10.) However, some of the larvae appeared to resist the bacteria, and although they appeared to be dead they were apparently sterile as far as could be observed from simple microscopical observations. The comb containing the infected larvae was then inserted in the nucleus.

Date of inoculation. 2 July.

Results. 2 days: All the infected larvae had been removed by the bees, and eggs laid in the empty cells. 5-8 days: No sign of any infected larvae. 10 days: Several of the larvae which the bees had reared in the introduced (inoculated) comb appeared to be sick, and two of these on examination proved to be full of *S. apis* cells. 11 days: One sick larva in the inoculated comb; this one full of *S. apis* cells. A very irregular appearance of the brood in this comb indicated that the bees had removed a very large number of larvae. 16 and 20 days: No affected larvae seen. 25 days: Thirty-three sick or dead larvae, mostly about 4 days old, were counted in the brood combs. Ten of these were examined microscopically. All of them contained masses of *S. apis* cells, together with a variable number of small rod-shaped bacteria, and in a few cases *B. alvei*-like organisms. *S. apis* (culture 4) was isolated from one of the recently

infected larvae. 29 days: Disease very marked and the nucleus was destroyed, the queen and twelve worker bees being used in Exp. 31. A very large number of infected larvae were counted in the brood combs. Thus there were approximately fifty-seven affected larvae in one of the combs adjacent to the one which was originally inoculated. Ten of these were examined. All showed *S. apis* cells in large numbers and a variable proportion of small rod-shaped bacteria, and some *B. alvei* cells. Two slimy evil-smelling larvae showed *B. alvei* cells and spores in apparently pure culture. *S. apis* (culture 6) and *B. alvei* (culture 14) were isolated in pure culture from remains of dead larvae.

EXP. 11.

Nucleus. Stores and sealed brood; eggs and young larvae; young larvae (inoculated comb); eggs, young larvae and stores; sealed brood and stores.

Inoculum. 425 and 530, 3-4-day-old larvae on either side of a comb were each fed 0.005 ml. of a suspension of *S. apis* cells, individual larvae receiving approximately 1.6×10^8 bacteria. The comb containing them was then incubated as in Exp. 10. At the conclusion of this time many of the larvae had been attacked by *S. apis* as in the previous experiment, and the comb was introduced into the nucleus.

Date of inoculation. 2 August.

Results. 4 days: Most of the infected larvae had been removed from the introduced comb and eggs laid in the empty cells. 6 days: No sign of infected larvae. 11 days: Several of the larvae which had been reared in the infected comb by the bees had been removed giving the brood an uneven appearance. One of seven larvae examined was full of *S. apis* cells, the remainder being unaffected. No infected larvae were seen on examinations of the brood 20, 27 and 30 days after inoculation.

The results of the above two experiments show that "European foul brood" can be initiated by placing a large number of larvae artificially infected with *S. apis* in a healthy nucleus, providing this is done early in the brood-rearing season and the nucleus is weak in bees. When the inoculation is carried out late in the brood-rearing season the bees are apparently able to ward off the disease. In the first experiment a mixed bacterial infection of the brood similar to "European foul brood" resulted when *S. apis* was employed alone in the infection of the starved larvae. However, as will be seen from Exp. 12, the relative proportion of *S. apis* cells in affected larvae was greater when *S. apis* was used to infect starved larvae than when *B. alvei* was used.

EXP. 12.

Nucleus. Stores; eggs and young larvae; young larvae (inoculated comb); eggs and stores. Weak in bees.

Inoculum. 180 and 395, 3-4-day-old larvae on either side of a comb were fed 0.005 ml. of a suspension of spores of *B. alvei*, each larvae receiving approximately 8.3×10^7 spores. The comb containing them was incubated as in previous experiments. At the conclusion of this time a very large proportion of the larvae were full of sporulating cells of *B. alvei*, though a few had apparently resisted the organism, and appeared sterile on ordinary microscopical examination. The comb containing these artificially infected larvae was then suspended in the nucleus.

Date of inoculation. 25 June.

Results. 2 days: Most, but not all, of the slimy larvae in the introduced comb had been removed by the bees, and eggs laid in the empty cells. 5 days: All the inoculated

larvae removed and no sign of infected larvae in any of the combs. 7 days: Young larvae being reared in the introduced comb, no sign of infection. 10 and 14 days: No sign of infection. 28 days: Fifty-one sick or dead larvae counted in the brood frames. Many of these were slimy and evil smelling. Ten were examined microscopically, and all showed *B. alvei* cells in large numbers, and the more recently infected larvae showed *S. apis* cells and a variable number of small rod-shaped bacteria. 32 days: The nucleus was destroyed, the queen being retained together with twelve worker bees for Exp. 31. Seventy-two sick or dead larvae were counted in one of the brood frames, and many of these were brown, slimy and evil smelling. Thus four slimy larvae of ten examined microscopically contained sporulating cells of *B. alvei* in apparently pure culture, the remainder having a large number of *B. alvei* and *S. apis* cells and a variable proportion of small rod-shaped bacteria. *S. apis* (culture 7) and *B. alvei* (culture 15) were isolated in pure culture from remains of dead larvae.

Exp. 13.

Nucleus. Sealed brood; eggs and young larvae; young larvae (inoculated comb); sealed brood; sealed brood.

Inoculum. 1000 and 900 3-4-day-old larvae on either side of a comb were fed 0.0025 ml. of a spore suspension of *B. alvei*, each larva receiving about 1.7×10^7 spores. The comb was then incubated as usual. At the end of this time many larvae were affected with the artificially induced disease and the comb containing them was inserted in the nucleus.

Date of inoculation. 9 July.

Results. 2 days: All infected larvae had been removed by the bees and eggs laid in the empty cells. Examinations 5, 7, 13 and 21 days after inoculation revealed no infection. 24 days: One suspicious-looking larva full of *S. apis* cells, and from this a pure culture (culture 3) of *S. apis* was isolated. Further examination of the brood 28, 32, and 34 days after inoculation revealed no infected larvae, and the experiment was concluded, since the nucleus had become queenless.

It is evident from the results of Exps. 12 and 13 that "European foul brood" can be initiated by placing a large number of larvae artificially infected with *B. alvei* in a healthy nucleus, provided this is done early in the brood-rearing season and that the nucleus is weak in bees. If the inoculation is carried out late in the brood-rearing season the bees are able to keep the disease in check. In the first experiment a "mixed infection" of the brood similar to "European foul brood" resulted when *B. alvei* alone was employed to infect the starved larvae. However, the relative proportion of *B. alvei* cells present in decaying larvae was much greater than in the experiment in which disease was induced employing a pure culture of *S. apis* (Exp. 11).

Exp. 14.

Nucleus. Sealed brood; eggs and young larvae; young larvae (inoculated comb); sealed brood. Strong in bees.

Inoculum. 720 and 945 2-4-day-old larvae on either side of a comb were fed 0.0025 ml. of a suspension containing a mixture of *S. apis* cells and *B. alvei* spores, each larva receiving approximately 1.4×10^6 spores of *B. alvei* and 1.6×10^6 vegetative cells of *S. apis*. The comb containing the inoculated larvae was incubated as usual. At the conclusion of the incubation period a large number of larvae had been attacked by the bacteria, and microscopical examination showed masses of *S. apis* cells and

B. alvei vegetative cells and spores in these. Some of the larvae were rather slimy, others were of a pasty consistency. The infected comb was then inserted in the nucleus.

Date of inoculation. 8 July.

Results. 2 days: All infected larvae had been removed from the introduced comb and eggs laid in the empty cells. 4 days: Eggs and healthy young coiled larvae in the introduced comb, no indication of disease. No infected larvae were observed during subsequent inspections of the brood 6, 7, 10, 11, 22, 25, 33, 35, 40 and 45 days after inoculation. By this time the nucleus was very strong in bees and breeding had almost ceased.

EXP. 15.

Nucleus. Stores and sealed brood; eggs and young larvae; young larvae (inoculated comb), eggs, young larvae and sealed brood. Very weak in bees.

Inoculum. 680 and 410 2-4-day-old larvae on either side of a comb were fed 0.0025 ml. of a mixed suspension of *S. apis* cells and *B. alvei* spores, each larva receiving approximately 9.1×10^7 cells of *S. apis* and 5×10^7 spores of *B. alvei*. The inoculated larvae were starved in the comb as usual, and then introduced in the customary manner to the nucleus.

Date of inoculation. 21 July.

Results. 3 days: Most of the dead larvae had been removed by the bees from the inoculated comb, and eggs laid in the empty cells. No infected larvae seen. 5 days: One dead larva in a comb adjacent to the introduced comb, and this was full of *S. apis* and *B. alvei* cells. 9 days: No infected larvae seen. 16 days: Two dead larvae full of *S. apis* and *B. alvei* cells. *B. alvei* (culture 18) was isolated in pure culture from one of these larvae. 18 and 20 days: No sick or dead larvae were seen. 22 days: Two sick larvae full of *S. apis* cells and small rod-shaped bacteria. 27 days: Four larvae full of *S. apis* cells and small rod-shaped bacteria. *S. apis* (culture 10) was isolated from one of these larvae. 29 days: Six sick or dead larvae full of *S. apis* cells and small rod-shaped bacteria. 36 and 40 days: Breeding very slowly, and no sign of any infected larvae.

It is apparent from the results of these two experiments that healthy nuclei are no more liable to contract disease when a mixture of *S. apis* and *B. alvei* is employed to initiate disease than when only one of these organisms is used. It can also be seen that, while a nucleus weak in bees can contract a transient form of "European foul brood" late in the brood-rearing season by the means of inoculation employed, a strong nucleus will not contract the disease even though it be inoculated somewhat earlier in the brood-rearing season.

EXP. 16.

Nucleus. Sealed brood and stores; young larvae and sealed brood; young larvae (inoculated comb); young larvae and sealed brood.

Inoculum. Forty larvae sick or dead of "European foul brood" were triturated in 20 ml. of sterile water. 530 and 350 2-4-day-old larvae on either side of a comb were fed 0.0025 ml. of this suspension. The comb containing the inoculated larvae was incubated in the usual manner, with the result that a large number of the larvae died with masses of *S. apis* and *B. alvei* cells in their tissues, and in addition some small rod-shaped bacteria. The comb was then introduced into the nucleus.

Date of inoculation. 20 July.

Results. 2 days: Most of the dead larvae in the introduced comb had been removed by the bees and eggs laid in the empty cells. No infected larvae were observed on examining the brood 4, 10, 13, 17, 21 and 23 days after inoculation. 28 days: One sick larva full of *S. apis* cells recovered, and from this a pure culture of *S. apis* (culture 11) was isolated. Subsequent inspections of the brood 37 and 45 days after inoculation revealed no infected larvae.

From this experiment it would appear that crushed suspensions of naturally infected "European foul brood" larvae are no more capable of initiating serious infection in the brood of a healthy nucleus relatively late in the brood-rearing season than are pure cultures of *S. apis* and *B. alvei* when the same technique is employed.

EXP. 17.

Nucleus. Stores; eggs, young larvae and sealed brood; young larvae (inoculated comb); eggs and sealed brood.

Inoculum. A Pasteur-Chamberland L 3 filtrate from European foul brood material was obtained by the technique previously described, forty larvae in all stages of the disease being crushed in 20 ml. of water for the purpose. A portion of the resulting filtrate was mixed with an equal volume of a suspension containing *S. apis* cells and *B. alvei* spores. 900 larvae 2-4 days old on one side of the comb, and 700 the other side, were fed 0.0025 ml. of this suspension, each larva receiving 0.00125 ml. of filtrate, 8.4×10^7 *S. apis* cells and 2.6×10^7 spores of *B. alvei*. The inoculated comb was incubated in the usual manner, many of the larvae decomposing with masses of *S. apis* and *B. alvei* cells in their tissues. It was then suspended in the experimental nucleus.

Date of inoculation. 19 July.

Results. 3 days: Nearly all the dead larvae in the inoculated comb had been removed by the bees, only a few brown scales remained. Eggs being laid in the empty cells. No infected larvae were seen during examinations of the brood 6, 11, 13, 18, 23, 28 and 37 days after inoculation. At the conclusion of this time, breeding was very slow.

This experiment shows that filtrate from infected brood mixed with the bacteria found in diseased larvae is no more capable of initiating disease relatively late in the brood-rearing season than are pure cultures of the bacteria alone. This may be looked upon as further evidence in favour of the fact that a virus is not implicated in the etiology of "European foul brood".

EXP. 18.

Nucleus. Young larvae and sealed brood; young larvae (starved without inoculation); eggs and young larvae; young larvae and sealed brood.

Inoculum. A comb containing a large number of 1-4-day-old larvae was selected, and was incubated as usual, the larvae not being inoculated. At the conclusion of the incubation period the comb contained many autolysing larvae, ten of which, on microscopical examination, appeared to be sterile. The comb was then inserted in the nucleus.

Date of inoculation. 27 June.

Results. 2 days: Most of the starved larvae had been removed by the bees and eggs laid in the empty cells. Further observations 4, 7, 11, 20, 22, 30, 36, 44, 49, 50 and 63 days after inoculation revealed no infection.

Exp. 19.

Nucleus. Stores; eggs and young larvae; eggs and young larvae; young larvae (starved); eggs and young larvae; sealed brood and stores. Very weak in bees.

Inoculum. 475 and 550 2-4-day-old larvae on either side of a comb were each fed 0.005 ml. of sterile brood filtrate. The comb was then incubated for 4 days in the customary manner. Microscopical examination of six autolysing larvae from the comb at the end of this time revealed no bacteria. It was then inserted in the nucleus.

Date of inoculation. 24 July.

Results. The dead larvae were removed by the bees in the usual manner and no infected larvae were observed 6, 14, 19, 24, 30, 40 and 49 days after inoculation.

Exps. 18 and 19 may be regarded as control experiments, for they show that uninfected starved larvae will not induce infection in healthy nuclei early or late in the brood-rearing season.

Exp. 20.

Nucleus. Eggs, young larvae and sealed brood; eggs, young larvae and sealed brood; inoculated comb; sealed brood.

Inoculum. A comb of freshly drawn foundation was selected, and each of 600 empty cells near the centre of the comb received 0.005 ml. of a suspension containing approximately 6.7×10^8 cells of *S. apis*. The comb was incubated 12 hours at 33° C., in order to dry the bacterial suspension, and it was then suspended in the nucleus.

Date of inoculation. 15 July.

Results. 10 days: A very even patch of sealed brood and young larvae had been reared by the bees on the inoculated side of the introduced comb, and no indication of any infection. No infected larvae were seen 15, 22, 26, 33 and 42 days after inoculation.

Exp. 21.

Nucleus. Stores; eggs and young larvae; inoculated comb; sealed brood; sealed brood and stores.

Inoculum. Approximately 2×10^8 spores of *B. alvei* were dried in each of 600 empty cells in a brood comb as in the previous experiment. The comb was then introduced into the nucleus.

Date of inoculation. 15 July.

Results. Eggs and young larvae almost ready for sealing were present in the inoculated comb and no infected larvae were seen. Examinations of the brood 15, 23, 28, 33 and 40 days after inoculation revealed no infected larvae.

From the results of the above two experiments it would appear that it is not possible to infect healthy nuclei relatively late in the brood-rearing season by the method employed.

Exp. 22.

(a) *Nucleus.* Stores; eggs and young larvae; eggs; young larvae and sealed brood; sealed brood and stores. Breeding relatively slowly.

Inoculum. *S. apis* was inoculated into 200 ml. of sterile milk containing 10 ml. of brood filtrate, and the resulting culture incubated for 12 hours at 35° C. The resulting coagulated milk was poured into the empty cells of the brood combs of the nucleus, some of it being poured over the coiled larvae.

Date of inoculation. 7 August.

Results. 3 days: Two suspicious-looking larvae; both of these contained a few *S. apis* cells. 5 days: No sign of infection. 10 days: One larva still living contained a few *S. apis* cells and small rod-shaped bacteria. No infected larvae were seen 19, 26 and 34 days after inoculation.

(b) *Nucleus.* Stores; eggs and sealed brood; eggs and young larvae; eggs and sealed brood; sealed brood and stores.

Inoculum. The method of inoculation was identical with that given above except that the milk culture was heated at 100° C. for 30 min. in order to kill the bacteria.

Date of inoculation. 7 August.

Results. No infected larvae were seen during examinations of the brood at the same intervals as recorded in the above experiment. This experiment was the control.

EXP. 23.

(a) *Nucleus.* Stores and sealed brood; eggs and young larvae; eggs and young larvae; eggs and stores. Breeding relatively slowly.

Inoculum. *B. alvei* was cultivated for 7 days at 35° C. in 250 ml. of milk containing 10 ml. of brood filtrate. At the end of this time 88×10^9 spores of *B. alvei* in 10 ml. of suspension were added to the milk culture in order to increase the inoculum. The culture was then poured over the brood frames of the nucleus.

Date of inoculation. 8 August.

Results. Examinations 2, 4, 9, 18, 24, 28 and 36 days after inoculation revealed no infected larvae.

(b) *Nucleus.* Sealed brood and stores; eggs and young larvae; young larvae; stores. Breeding relatively slowly.

Inoculum. The inoculum was identical with that employed in the first part of the experiment except that the milk culture was autoclaved in order to kill the spores of *B. alvei*.

Date of inoculation. 8 August.

Results. No infected larvae were seen at intervals of examination identical with those employed in part (a) of the experiment.

The results of Exps. 22 and 23 indicate that it is not possible to infect healthy nuclei with milk cultures of *S. apis* or *B. alvei* late in the brood-rearing season. The experiments should be repeated early in the brood-rearing season.

EXP. 24.

Nucleus. Stores; sealed brood and young larvae; eggs and young larvae (inoculated); eggs and stores; sealed brood.

Inoculum. 50 ml. of a suspension of *S. apis* cells containing approximately 1.4×10^{13} organisms was poured over the eggs and young larvae of one of the brood combs. The comb was then suspended in the nucleus.

Date of inoculation. 22 July.

Results. 3 days: A fairly large number of the larvae in the inoculated comb had been removed by the bees as was evidenced by a pronounced irregularity in distribution of the brood. Further examinations 8, 11, 16, 21, 26, 35 and 42 days after inoculation revealed no infected larvae.

This experiment indicates that disease cannot be induced in a healthy nucleus fairly late in the brood-rearing season by pouring relatively large numbers of *S. apis* vegetative cells over young coiled larvae.

*II. Inoculation of experimental nuclei by feeding the bees suspensions
containing bacteria or other infective material*

EXP. 25.

Nucleus. Stores; sealed brood; eggs; young larvae and eggs.

Inoculum. Forty larvae sick or dead of "European foul brood" were ground up with 50 ml. of sterile syrup and fed to the bees of the nucleus.

Date of inoculation. 10 July.

Results. 2-days: All the infective material had been taken by the bees. 5 days: Three sick larvae seen. 9 days: Four sick larvae in one comb. All of these had *S. apis* cells in large numbers, and two of them small rod-shaped bacteria as well. 13 days: Six affected larvae were recovered. All of these contained large numbers of *S. apis* cells and a variable proportion of small rod-shaped bacteria. *B. alvei* cells were seen in one of the affected larvae. Pure cultures of *S. apis* (culture 5) and of *B. alvei* (culture 13) were isolated. 22 days: Four larvae full of *S. apis* cells and small rod-shaped bacteria were recovered. One of these contained a fair number of *B. alvei* cells. 33 days: One affected larva full of *S. apis* cells recovered. Subsequent examinations of the brood 45, 51, 55 and 62 days after inoculation revealed no further infection.

This experiment is of interest in that it shows that a nucleus of hybrid bees is capable of keeping "European foul brood" in check, even when a relatively large inoculum of infectious material from natural sources is given, providing that it is given at a time when brood rearing is about to decline.

EXP. 26.

Nucleus. Eggs; young larvae; stores. Weak in bees.

Inoculum. The bees were fed infectious material on four successive occasions as follows:

1st day:	34×10^{10}	vegetative cells of <i>S. apis</i>	is	50 ml. of syrup.
3rd day:	44×10^{10}	"	"	"
5th day:	52×10^{10}	"	"	"
19th day:	46×10^{10}	"	"	"

Date of first inoculation. 8 July.

Results. Inspections of the brood up to the time of the last inoculation, and thereafter at intervals of 28, 36, 40, 47, 56 and 66 days after the first inoculation, revealed no infected larvae.

EXP. 27.

Nucleus. Eggs; young larvae; stores. Weak in bees.

Inoculum. The bees were fed infectious material on three successive occasions as follows:

1st day:	4×10^{11}	spores of <i>B. alvei</i>	in	50 ml. of syrup
8th day:	4.6×10^{11}	"	"	"
10th day:	5.1×10^{11}	"	"	"

Date of first inoculation. 2 July.

Results. No infected larvae were observed up to the time of the last feeding, nor at intervals of 16, 23, 32, 36, 41, 46 and 64 days after the first feeding.

From the results of these experiments it would seem that nuclei are not infected by feeding the bees large numbers of *S. apis* vegetative cells or *B. alvei* spores, even when several successive doses are fed.

Exp. 28.

Nucleus. Sealed brood and stores; eggs, young larvae and sealed brood; drawn comb; eggs, young larvae and sealed brood; stores and sealed brood.

Inoculum. 20 ml. of a Pasteur-Chamberland L 3 filtrate prepared in the usual manner from 100 larvae sick or dead of "European foul brood" crushed in 30 ml. of sterile brood filtrate were fed to the bees of the nucleus.

Date of inoculation. 19 July.

Results. No infection was observed 3, 6, 11, 22, 29, 37 and 45 days after inoculation.

Exp. 29.

Nucleus. Eggs and young larvae; sealed brood and stores; sealed brood and stores; eggs and young larvae; sealed brood and stores.

Inoculum. 25 ml. of a Pasteur-Chamberland L 2 filtrate prepared in the usual manner from ninety "European foul brood" larvae in all stages of disease crushed in 50 ml. of sterile brood filtrate were fed to the bees of the nucleus.

Date of inoculation. 22 July.

Results. No infected larvae were seen 3, 9, 15, 21, 26, 33 and 42 days after inoculation.

Exp. 30.

Nucleus. Stores; eggs and young larvae; sealed brood; stores; eggs and young larvae.

Inoculum. The bees were fed infectious material on each of two successive days as follows:

First feeding: 2×10^{10} spores of *B. alvei*; 6.7×10^{11} vegetative cells of *S. apis*; 5 ml. of a Pasteur-Chamberland L 2 filtrate, prepared as in Exp. 29; and 15 ml. of sterile syrup.

Second feeding: 2×10^{11} spores of *B. alvei*; 3.7×10^{11} vegetative cells of *S. apis*; 5 ml. of the Pasteur-Chamberland L 2 filtrate; and 15 ml. of sterile syrup.

Date of inoculation. 15 July.

Results. No infected larvae were found when the brood was examined 4, 8, 18, 24, 33, 35, 40 and 43 days after inoculation.

The above three experiments may be regarded as additional evidence in favour of the fact that a virus is not implicated in the etiology of "European foul brood".

III. *The results of transferring queens from nuclei affected with "European foul brood" to healthy queenless nuclei*

Exp. 31.

The queens from the nuclei used in Exps. 2, 10 and 12 were kept for 48 hours in queen cages with twelve worker bees; the cages containing candy as usual. Three three-comb nuclei (two combs of sealed brood and stores and one frame with foundation) were made up, and one of the above queen bees was introduced into each nucleus, the date of introduction being 1 or 2 August.

Results. 9 days after introduction: Large numbers of eggs had been laid in at least two frames of each nucleus. 16 days: Eggs, young larvae and sealed brood in each nucleus, and no sign of any infected larvae. Further inspections of the brood 23, 29 and 41 days after the introduction of the queens revealed no infected larvae.

This experiment may be regarded as further evidence in favour of the fact that "European foul brood" is not carried by the queen.

DISCUSSION

It is evident from the results of the experiments described in this paper that "European foul brood" can be initiated in healthy nuclei by feeding either the bees or the larvae direct naturally infected material. However, the success attendant upon such experiments varies greatly according to the conditions under which they are carried out. Thus disease was readily induced in a nucleus weak in bees in the active part of the brood-rearing season by inserting in it a comb containing many larvae affected with "European foul brood". When small amounts of a suspension of crushed "European foul brood" larvae were fed direct to larvae rather early in the brood-rearing season different results were obtained in each of two experiments. In one case the disease appeared but was merely of a mild transient form, never assuming serious proportions, and disappearing toward the end of the brood-rearing season. In the other case no disease resulted; probably because the nucleus was strong in bees of a good "house-cleaning" type. When forty larvae affected with "European foul brood" were fed in syrup to the bees of a healthy nucleus about half-way through the brood-rearing season, disease resulted but was not of a serious kind, and toward the end of the brood-rearing season no sick or dead larvae could be found. In a further experiment small amounts of crushed "European foul brood" larvae were fed directly to young coiled larvae, the comb containing them being incubated for 4 days at 35° C. The comb containing these was then introduced into a healthy nucleus rather late in the brood-rearing season, with the result that no disease developed.

Experiments have also shown that "European foul brood" can be induced in healthy nuclei by inserting in them combs containing larvae which have been artificially infected by feeding them pure cultures of *B. alvei* or *S. apis* and starving them for 4 days at 35° C. The susceptibility of the larvae of healthy nuclei to infection when combs containing larvae thus infected are inserted in them appears to be governed by approximately the same conditions as pertain to inoculations made with infected larvae obtained from natural sources. Thus, in the experiments

recorded, inoculation of nuclei by this means early in the brood-rearing season was followed by a severe infection, while later in the season either a very mild transient infection resulted, or no disease was produced. It is of some interest that even when a pure culture of *S. apis* or *B. alvei* was used to infect the larvae which were employed in the starvation experiments the disease which ultimately resulted was a mixed one in which both these bacteria were recovered from certain of the infected larvae. However, the relative proportion of *B. alvei* and *S. apis* varied, depending on which of them had been employed in the original inoculations. Thus, in the case of an experiment made using starved larvae which had been infected with *S. apis*, the number of larvae with masses of sporulating *B. alvei* cells was less than in the case of a similar experiment in which *B. alvei* had been employed to inoculate the larvae prior to starvation. In further experiments a mixture of *B. alvei* and *S. apis* was fed to larvae which were subsequently starved in their combs and then introduced into healthy nuclei. In one experiment a mild transient form of "European foul brood" resulted when a nucleus very weak in bees was inoculated in this manner quite late in the brood-rearing season. In another experiment in which a nucleus of normal strength was inoculated much earlier in the brood-rearing season no disease resulted. Certainly it would appear from these results that a mixture of *S. apis* and *B. alvei* cells is no more capable of initiating disease than one of these organisms alone by the technique employed. Control experiments in which uninfected starved larvae were introduced into nuclei early and late in the brood-rearing season were unsuccessful as regards initiating disease.

It is of some interest that a mixed bacterial infection of the brood ultimately resulted when pure cultures of *B. alvei* or *S. apis* were used to infect larvae by the "starved larvae" technique. The reason for this is not known. It does seem possible that the bees introduce bacteria into recently infected or decaying larvae. Certainly bees are by no means sterile creatures bacteriologically, and Bruce White(22) found that normal healthy bees carry an organism similar to *S. apis* in their intestinal tracts. It is well known that the normal hive contains certain spore-forming bacteria, including *B. alvei*. In general the results of these experiments suggest that "European foul brood" may not be a single disease, but is, perhaps, a mixed bacterial infection of the brood of weak colonies of bees. However, any absolute conclusion regarding this must be postponed until additional experiments can be carried out.

Up to the present time it has not been found possible to infect the

brood of healthy nuclei by feeding the bees several successive doses containing either *S. apis* vegetative cells or *B. alvei* spores in large numbers. Likewise no disease has been initiated when larvae have been fed these organisms directly and then immediately placed in healthy nuclei without a preliminary starvation period. In this respect the results obtained by Borchert^(5, 6) have not been verified. The reason for this is not clear, but it is possible that the race of bee used by Borchert was one which was very susceptible to disease of the "European foul brood" type. Attempts to cause infection by pouring milk cultures *S. apis* or *B. alvei* over developing brood of healthy nuclei have also proved unsuccessful; however, these experiments have been carried out rather late in the brood-rearing season. Bacteria-free filtrates prepared by filtering crushed suspensions of "European foul brood" larvae through Pasteur-Chamberland L 2 or L 3 filters have not caused infection when fed directly to the larvae or to the bees of healthy nuclei, either alone or in combination with bacteria. From these results it would appear permissible to conclude that a filterable virus is in no way implicated in the etiology of this type of brood infection, especially in view of the successful results obtained with pure cultures of bacteria.

The results of all the experiments outlined above indicate that, with the type of bee used, it is normally not easy to initiate "European foul brood" in healthy nuclei unless inoculations are made early in the brood-rearing season, that is at a time when the preponderance of young larvae over both "nurse" and "house-cleaning" bees is relatively high, and consequently at a time when the larvae may be subject to a condition approaching malnutrition. This finding is in direct agreement with the important observations made by Sturtevant⁽¹³⁾ with reference to the conditions favouring the spread of "European foul brood". This investigator found that black and hybrid bees are more susceptible to "European foul brood" than are Italian races; that the disease normally occurs only during the early part of the brood-rearing season; that Italian stocks of normal strength will not contract the disease; that the disease frequently disappears when an affected stock experiences a good honey flow and the season advances; and that it is relatively easy to infect a weak stock of black or hybrid bees in the spring, but that it becomes increasingly difficult, if not impossible, to infect such a stock during a heavy honey flow late in the summer when breeding is slow.

At present no really satisfactory explanation can be advanced for the fact that such a large inoculum of infected starved larvae is required to induce disease, and that bacteria fed directly to bees or to larvae

which are subsequently attended to by the "nurse bees" have not, in the experiments recorded in this paper, caused disease. Perhaps a considerable attenuation with respect to virulence follows when the bacteria are cultivated for even a short time upon laboratory media, and passage through larvae whose resistance has been weakened by starvation may restore virulence. Again the possible influence of decomposing brood as a convenient "vehicle" for transmitting the disease must be considered, as well as the fact that the initial inoculum is probably greater when the "starved larvae" technique is employed than when bacterial suspensions are fed.

The cause of so-called "European foul brood" has long been a matter of controversy. White^(19, 20) believed that a microorganism, *B. pluton*, which could not be cultivated on any medium studied caused this disease. Lochhead^(9, 10) suggested that *B. pluton* did not exist as an organism distinct from *S. apis*, and that the forms seen by White, and which were called *B. pluton* by him, were merely lanceolate-shaped vegetative cells of *S. apis*. He also concluded, on the basis of certain morphological observations, that "European foul brood" might be caused by a pleomorphic organism which could assume the form of *S. apis* or *B. alvei*. This view has recently been adopted by Burnside^(7, 8), who apparently believes that *S. apis*, *B. alvei* and *Bacterium eurydice* may all be forms of a single pleomorphic organism. Since this worker has also suggested that so-called *B. para-alvei* may dissociate into *S. apis* one is forced to conclude, on this theory, that all four organisms are pleomorphic forms of a single organism. While this view has not been disproved as yet the evidence advanced in favour of it is incomplete, and there is room for further investigation. Borchert^(1, 2, 3, 4, 5, 6) has recently advanced certain evidence in favour of the fact that so-called "European foul brood" is not a single disease, but merely represents a mixed bacterial infection of the brood. This explanation seems a fairly plausible one in view of the facts already recorded in this paper. Moreover, the disease varies considerably as regards bacterial flora. Thus several different species of spore-forming bacilli have been described as occurring in different cases of this type of infection^(5, 6, 16). Also mixed bacterial diseases of the brood other than the disease described as "European foul brood" by White^(19, 20) have been noticed^(8, 17, 18).

The results obtained by certain other investigators^(3, 4, 5, 7, 9, 10) have led them to assume that *B. pluton* White does not exist as a species distinct from *S. apis*, but perhaps this conclusion should not yet be regarded as final. Certainly the lanceolate-shaped coccus cells seen in larvae

affected with "European foul brood" are apparently not very different from those encountered in pure cultures of *S. apis* or those seen in larvae artificially infected with this organism employing the "starvation technique" (Pl. XXII, fig. 6 and Pl. XXIII, figs. 7-12). It is not unlikely that so-called "European foul brood" is no well-defined single disease. Rather would it appear that it may be a mixed bacterial infection of the brood of bees occurring in the main in the brood of weak colonies, and that it has no single specific etiological agent. The theory that the bacteria found in "European foul brood" are pleomorphic forms of a single species must be regarded with caution until more experimental work has been carried out. As yet the evidence presented in support of this hypothesis is by no means adequate enough to warrant its adoption.

SUMMARY

"European foul brood" has been initiated in healthy nuclei by feeding either the young larvae directly or the bees naturally infected material. The disease thus produced varied from a mild transient infection to a serious form depending upon whether the inoculation had been made early or late in the brood-rearing season. In one experiment in which a nucleus very strong in bees was employed no disease resulted.

"European foul brood" has also been induced in healthy nuclei by suspending in them combs containing artificially infected larvae in which disease had been produced by feeding them pure cultures of *Streptococcus apis* or *Bacillus alvei* and then starving them under conditions favouring the growth of the bacteria. The susceptibility of nuclei to infection by this means seems to be governed by approximately the same conditions as pertain to inoculations made with material obtained from natural sources. The disease thus induced ultimately becomes a mixed bacterial infection of the brood.

Attempts to cause disease by feeding the bees or larvae (without starving them) relatively large numbers of *S. apis* or *B. alvei* organisms have as yet proved unsuccessful. Whether this is due to the fact that these bacteria become attenuated with respect to virulence by culturing them on artificial media, or that decomposing brood acts as a vehicle and that the relative inoculum is greater by this method, remains to be determined.

The failure to produce "European foul brood" by feeding sterile Pasteur-Chamberland L2 or L3 filtrates prepared from naturally infected larvae to the bees or larvae of healthy nuclei, either with or without bacteria, may be taken as strong evidence in support of the

view that a filterable virus is in no way implicated as an etiological agent in this type of disease. This conclusion is strengthened by the success which has attended the use of pure cultures of the bacteria associated with this disease employing the "starved larvae" technique described.

The introduction of queen bees from nuclei affected with "European foul brood" into healthy queenless nuclei has not caused any transmission of the disease under the conditions of the experiments.

Two species of *S. apis* Maassen have been isolated from affected larvae taken from several different cases of "European foul brood"; one of these hydrolyses both casein and gelatine, the other does not. In other respects these species appear to be identical.

The etiology of so-called "European foul brood" is discussed in detail, and it is suggested from the evidence submitted in this and other papers that it may not be a single disease with one well-defined etiological agent, as is American foul brood, but is, perhaps, a non-specific mixed bacterial infection of the brood of bees, especially of the brood of weak colonies. This conclusion must be regarded as temporary pending further investigation.

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EXPLANATION OF PLATES XXII-XXIV

(All photographs $\times 1200$)

PLATE XXII

- Fig. 1. *Streptococcus apis* (culture 1) after 16 hours' growth on beef digest agar. This strain was a gelatin and casein hydrolyser and was employed in all the infection experiments.
- Fig. 2. *Streptococcus apis* (culture 3) after 24 hours' growth on beef-digest agar. Note the chain of cells. This strain was not a casein or gelatin hydrolyser.
- Fig. 3. *Streptococcus apis* (culture 3) after 24 hours' growth on Lochhead's sucrose-glucose-peptone agar (10).
- Fig. 4. *Streptococcus apis* (culture 1) after 16 hours' growth on Lochhead's sucrose-glucose-peptone agar (10).
- Fig. 5. *Streptococcus apis* (culture 1) after 12 hours' growth on beef-digest agar containing 1 ml. of M/5 phosphate buffer pH 7.0 per 5 ml. Note the very variable size of the cells.
- Fig. 6. *Streptococcus apis* growing in the tissues of a starved larva after 4 days at 35° C. Note the lanceolate-shaped cells.

PLATE XXIII

- Fig. 7. *Streptococcus apis* and *Bacillus alvei* (a single vegetative cell) growing in a larva sick of "European foul brood".
- Fig. 8. *Streptococcus apis* showing a mass of typical lanceolate-shaped cells in a larva affected with "European foul brood". A few small rod-shaped bacteria (*Bacterium eurydice*?) can also be seen.
- Fig. 9. *Streptococcus apis* and *Bacillus alvei* growing in the tissues of a larva which had been fed a mixed suspension of the cells of both these organisms and then starved for 4 days at 35° C.
- Fig. 10. *Streptococcus apis* and *Bacillus alvei* growing in the tissues of a "starved larva".
- Fig. 11. *Streptococcus apis*, *Bacillus alvei* and a few small rod-shaped bacteria (*Bacterium eurydice*?) growing in the tissues of a larva affected with "European foul brood". This larva was one of those recovered in Exp. 15.
- Fig. 12. A preparation similar to that shown in Fig. 11, only from another case of "European foul brood".

PLATE XXIV

- Fig. 13. *Bacillus alvei* growing in a "starved larva". This larva was brown, slimy and rather evil smelling as a naturally infected larva. Note the spore formation.
- Fig. 14. *Bacillus alvei* in a slimy decaying "European foul brood" larva. The sporulating vegetative cells arranged round the large fat body are similar to those shown in Fig. 13.
- Fig. 15. *Bacillus alvei* and *Streptococcus apis* in a larva dead of "European foul brood".
- Fig. 16. *Streptococcus apis* and a torula or yeast in a larva dead of an atypical "European foul brood". In this case the torula or yeast appeared to take the place normally occupied by *Bacillus alvei* (17).

Note. In most of the preparations made from affected larvae a greater or lesser number of round fat bodies of variable size are to be seen; e.g. Figs. 6, 8, 9, 10, 12 and 13.

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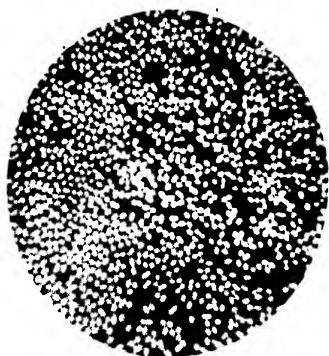


Fig. 1

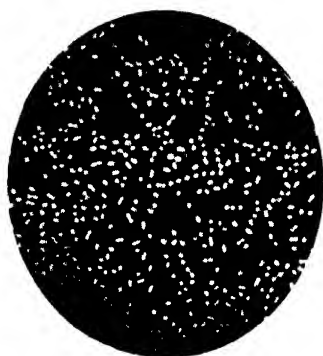


Fig. 2

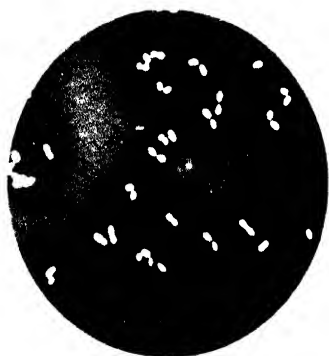


Fig. 3

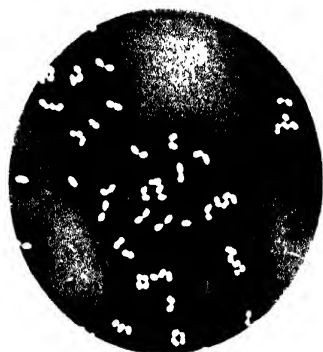


Fig. 4

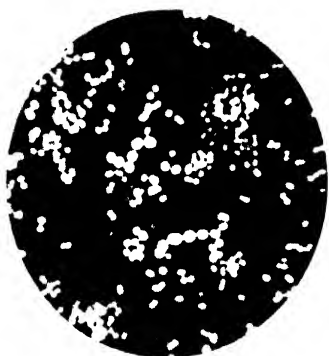


Fig. 5



Fig. 6



Fig. 7

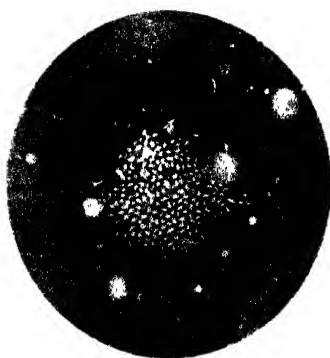


Fig. 8



Fig. 9



Fig. 10



Fig. 11



Fig. 12

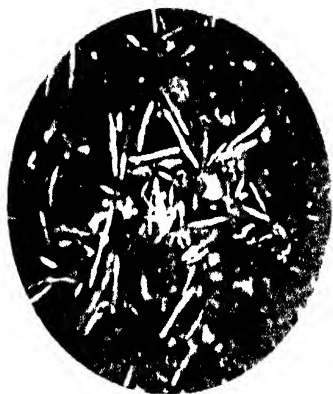


Fig. 13



Fig. 14



Fig. 15

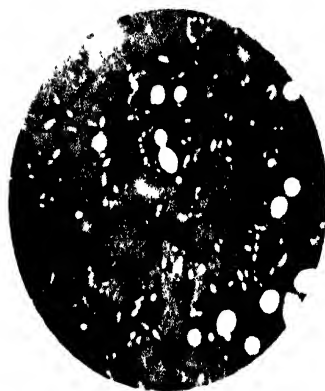


Fig. 16

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STUDIES ON EUROPEAN FOUL BROOD OF BEES

III. FURTHER EXPERIMENTS ON THE PRODUCTION OF THE DISEASE

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(With Plate XXXIX)

IN a previous communication (5) the results of experiments on European foul brood were recorded. It was found that certain of the bacteria commonly occurring in larvae affected with the disease (*Streptococcus apis* and *Bacillus alvei*) were incapable of producing it when fed in large numbers to the bees or larvae of healthy colonies. The disease was initiated by introducing infected material obtained from natural sources into healthy nuclei, or by suspending in them larvae which had been artificially infected with *Streptococcus apis* or *Bacillus alvei* employing a special technique. The facts that European foul brood usually appears in weak colonies of bees in the spring, and that it tends to disappear with the advent of a honey flow and a decrease in brood-rearing activity were verified.

The results of these experiments were not sufficiently conclusive to implicate any single organism as the primary etiological agent in European foul brood, and it was temporarily suggested that the disease might merely be a mixed bacterial infection of the brood of weak colonies of bees. Experiments to be described in this paper have shown that this view was erroneous, for careful work has resulted in a verification of the findings of White (9, 10), namely that a micro-organism, which has so far resisted all attempts to cultivate it *in vitro*, probably causes the disease. The experimental basis for this conclusion will be outlined.

THE MORPHOLOGY OF THE PRIMARY INVADING ORGANISM IN EUROPEAN FOUL BROOD: ITS DISTINCTION FROM SO-CALLED *STREPTOCOCCUS APIS*

White (9, 10) stated that European foul brood was a single disease caused by a lanceolate-shaped coccus which he named *Bacillus pluton*. The organism failed to multiply on a number of different media which he studied, and his findings have, therefore, been much criticized. Thus

Lochhead, Burnside & Borchert, whose work has been cited in previous papers (4, 5), and, formerly, the writer have all rejected this view. The remarkable confusion which has arisen with reference to the cause of this disease is largely due to the failure of these investigators to differentiate *B. pluton* from the so-called "*Streptococcus apis*" species which so frequently occur in affected larvae. *S. apis* can no longer be considered a distinct species, for the lactic acid streptococci which often occur in larvae affected with European foul brood are apparently identical with *S. liquefaciens* and *S. glycerinaceus* (1, 7). These species commonly occur in cattle faeces and may be collected by the bees when seeking moisture. Though it is strictly incorrect, the designation *S. apis* will be retained for convenience when referring to the lactic acid streptococci which occur in larvae affected with European foul brood.

In carrying out experiments on the production of European foul brood in 1935 the writer noticed that numerous lanceolate-shaped coccus organisms were present in the intestines of certain larvae examined, and that no growth of these cells occurred when media suitable for cultivating *S. apis* were used. At the time little credence was given to this observation, partly in view of the fact that a large body of published data had recently appeared stating that *Bacillus pluton* did not exist as a species distinct from *Streptococcus apis*, and partly because it was believed, erroneously, that these cocci were merely *S. apis* organisms which had either undergone autolysis, or had been killed by the larvae. For these reasons the mistake of referring to all the coccus-shaped organisms seen in larvae affected with European foul brood as *S. apis* was made, and it is fairly certain that the cocci shown in Figs. 8, 11, 12, 15 and 16 of the last paper of this series (5) were *Bacillus pluton* and not *Streptococcus apis*. *Bacillus pluton* and *Streptococcus apis* can be distinguished morphologically, especially in unstained preparations, though this distinction is not always a very sharp one. This difference is best shown by a series of photomicrographs (Pl. XXXIX). In Fig. 1 *Bacillus pluton*, as it appears in the intestine of an apparently healthy larva about 3 days old taken from a colony of bees affected with European foul brood, is shown. In this figure the organisms are embedded in a fold of membrane; probably this is a portion of the peritrophic membrane, for White (10) stated that *B. pluton* is localized there. At this stage the cocci are invariably rather small and occur chiefly in pairs and, very occasionally, in short chains but, as they increase in number, they tend to occur singly. This transition is shown in Fig. 2, short chains and pairs of cocci, and oval cocci occurring singly in an apparently healthy larva about 3 days old being illustrated.

B. pluton, as it occurs in larvae about 4 days old definitely affected with European foul brood but still living, is shown in Figs. 3 and 4. These cocci have a characteristic oval shape with pointed ends, are of variable size, and frequently occur in clumps as shown in Fig. 3. *B. pluton* and *Streptococcus apis*, as they occur together in larvae affected with European foul brood, are shown in Figs. 5 and 6. In both these preparations the typical lanceolate-shaped cells of *Bacillus pluton* can be distinguished from the more spherical cells of *Streptococcus apis*. The small rod-shaped bacteria shown in Figs. 3-6 are the *Bacterium eurydice* of White. *Streptococcus apis* was readily cultured from the stomachs of the larvae used in making the preparations for Figs. 5 and 6, but all attempts to cultivate the cocci from the material used for the preparations from which Figs. 1-4 were made failed. It must be concluded that *Bacillus pluton* and *Streptococcus apis* are not identical organisms.

From the marked variation in the type of coccus cell shown in Figs. 1-4 it might be inferred that more than one species of coccus which cannot be cultured *in vitro* occurs in larvae affected with European foul brood, that is, that *Bacillus pluton* is not a single species. Naturally this question must remain unanswered until such time as the organisms are cultivated and identified. Meanwhile it would seem wise to employ the somewhat unsuitable designation *B. pluton* for them.

EXPERIMENTAL

The technique employed in the identification and cultivation of the bacteria used was identical with that previously described (5). Suspensions of the spores of *B. para-alvei* (6), *B. orpheus* and of a recently described bacillus (Culture 1) (4) were prepared similarly to those of *B. alvei*. The strain of *Streptococcus apis* employed was a gelatin liquefier (*S. liquefaciens*) isolated from a larva affected with European foul brood. The method of preparing and inoculating experimental nuclei was the same as that previously described (5). A De Vilbiss No. 15 atomizer (sterilizable) was used for spraying suspensions of bacteria over developing brood. Since the causal organism of European foul brood has not been isolated, the presence of the disease in inoculated nuclei was diagnosed by the external appearance of affected larvae and by a microscopical examination of a number of them. In experiments in which a modification of the disease was induced by spraying suspensions of certain of the secondary invading micro-organisms (*Bacillus para-alvei*, *B. orpheus*, etc.), diagnosis involved both a microscopical examination of

several infected larvae, and the isolation of the secondary invading organism from one of them.

Suspensions of *B. pluton* for infection experiments were made by removing, with aseptic precautions, the intestines from a large number of apparently healthy or very slightly affected larvae taken from brood combs of colonies badly affected with European foul brood. Almost invariably a small proportion of these contain *B. pluton* in apparently pure culture (as shown in Figs. 1-3); and a fairly uniform suspension of the organism can be obtained by triturating the whole intestine, or portions of it, in sterile *M*/5 phosphate buffer pH 7.2. The approximate number of vegetative cells in such suspensions can be estimated by means of a Thoma haemocytometer slide.

The examination of large numbers of apparently healthy or slightly affected larvae from colonies affected with European foul brood has shown that *B. pluton* occasionally occurs in the first named group, and is invariably present in the latter. *Bacterium eurydice* is usually the next organism to appear, and as the disease advances such bacteria as *Streptococcus apis*, *Bacillus alvei* or other putrefactive organisms are most prevalent.

Attempts to cultivate Bacillus pluton

Material for the attempted isolation of this species must be derived from very recently infected larvae in which the organism occurs in apparently pure culture in the intestinal tract. Occasionally, apparently uncontaminated suspensions of *B. pluton* are obtained and, in such cases, no multiplication of the cocci occurs. More frequently, however, a few cells of *Bacterium eurydice* are present in such preparations and, although they are not noticed on microscopical examination, these soon multiply and obscure the cocci. Media commonly employed in cultivating the bacteria associated with brood diseases (5, 8), when used with or without the addition of broad filtrate (8), would not support growth of *Bacillus pluton*. "Royal jelly" alone (which is practically sterile bacteriologically) and sterile Seitz filtrates prepared from neutralized extracts of this substance were tried without success. Tryptic digests or autolysates of whole drone or worker larvae (prepared over chloroform), or similar preparations made from washed larval intestines alone, would not support growth either anaerobically or aerobically. In these experiments a pH of 7.0-7.2 was usually employed, but lower pH values have proved equally unsuitable. Since most of these media were sterilized by means of a Seitz filter it is possible that some essential growth factor was removed.

It was previously shown (5) that *B. alvei* and *Streptococcus apis* would grow in 3-4-days-old larvae when fed to them, and the possibility that *Bacillus pluton* could be cultivated in this manner was considered. However, when relatively large doses of *B. pluton* from the intestines of infected larvae were fed to healthy 3-4-days-old larvae, no growth occurred. *B. pluton* organisms could, microscopically, be demonstrated readily in the stomachs of the recently fed larvae, but they rapidly decreased in numbers and in most cases practically or entirely disappeared, the larvae dying and autolysing, occasionally with no visible bacteria in their intestinal tracts, but more frequently with numbers of cells of *Bacterium eurydice*. This finding is not entirely unexpected since 3-4-day-old larvae possess a strong bactericidal mechanism (5), frequently destroying vegetative cells of *Streptococcus apis* and spores of *Bacillus alvei*, especially when moderate doses of these organisms are fed to them. It seems probable that *B. pluton* is a strict parasite, is introduced into very young larvae by the bees, perhaps at the time of hatching from the egg, and that once established it multiplies rapidly in the intestine, probably being localized in the peritrophic membrane, where a delicate balance of nutrients favouring its growth is reached.

*The production of European foul brood with suspensions
of Bacillus pluton*

In order to confirm and extend the results of previous experiments (5) attempts to produce European foul brood were made by spraying very heavy suspensions of the vegetative cells of *Streptococcus apis* and *Bacterium eurydice*, and the spores of *Bacillus alvei* and *B. para-alvei*, over the developing brood of healthy nuclei early in the brood-rearing season. Disease was not initiated in any of these experiments. On the other hand European foul brood was again produced with *Streptococcus apis* and *Bacillus alvei* employing the starved larva technique previously described (5). The following experiments show that relatively small doses of *B. pluton* organisms, obtained from the intestines of affected larvae, will readily induce European foul brood early in the brood-rearing season.

Exp. 1.

The intestines of three recently infected, apparently healthy larvae, in which *B. pluton* occurred in apparently pure culture, were triturated with 25 ml. of sterile M/5 phosphate buffer pH 7.2. The resulting fairly uniform suspension of bacteria, together with a certain amount of cell debris, was centrifuged for 15 min. in order to concentrate most of the bacterial cells. The supernatant fluid (a), which was almost free from bacterial cells, was carefully decanted, and the precipitate (b), containing

most of the *B. pluton* organisms, was suspended in 25 ml. of the above phosphate buffer. Two different nuclei were inoculated with these suspensions as follows:

Nucleus 1. Eggs and stores; young larvae and sealed brood; eggs and young larvae (inoculated).

Inoculum. The eggs and young larvae in the comb indicated were sprayed with the supernatant liquid (a).

Date of inoculation. 10 July.

Results. Examination of the brood 3, 6, 12, 17, 19, 26, 41 and 57 days after inoculation revealed no infected larvae.

Nucleus 2. Eggs and stores; eggs and young larvae (inoculated); eggs and stores.

Inoculum. The above suspension (b) containing approximately 72×10^7 cells of *B. pluton*, was sprayed over the developing brood of the comb indicated.

Date of inoculation. 10 July.

Results. 3 days: Five infected larvae seen in the inoculated comb; all showed large numbers of *B. pluton* cells in their intestines and *Bacterium eurydice* as well. 6 days: Ten infected larvae removed; all showed *Bacillus pluton* and small rod-shaped cells of *Bacterium eurydice* in their intestinal tracts. Subsequent examinations 12, 17, 19 and 26 days after inoculation revealed very large numbers of infected larvae. The nucleus was used for Exp. 4 (*vide infra*).

This experiment shows that cell free extracts prepared from intestines of larvae affected with European foul brood do not cause the disease, while the cells of *Bacillus pluton* soon cause infection. These results substantiate those of previous experiments which indicated that a filterable virus is not implicated as etiological agent in this disease (5).

EXP. 2.

A fairly uniform suspension of *B. pluton* cells in M/5 sterile phosphate buffer, containing approximately 98×10^6 organisms per ml., was prepared as previously described, and the bees of three different nuclei were fed various amounts in 30 ml. of syrup as follows:

Nucleus 1. Eggs and young larvae; eggs and young larvae.

Inoculum. Approximately 98×10^6 cells of *B. pluton* were fed to the bees.

Date of inoculation. 25 June.

Results. No diseased larvae were seen on examination of the brood 6, 7, 12, 18, 21, 32, 41 and 56 days after inoculation.

Nucleus 2. Eggs and stores; eggs and young larvae; eggs and young larvae.

Inoculum. Approximately 490×10^6 cells of *B. pluton* were fed to the bees.

Date of inoculation. 25 June.

Results. 6, 7 and 12 days: No infected larvae seen. 18 days: Two affected larvae seen and examined. Both were full of *B. pluton* organisms and also *Bacterium eurydice*. 21 days: The disease had spread rapidly and ten affected larvae were counted in two brood combs. Six of these examined showed *Bacillus pluton* cells and varying numbers of *Bacterium eurydice* organisms in their intestines. The nucleus was subsequently used for Exp. 5 (*vide infra*).

Nucleus 3. Eggs and stores; eggs and young larvae; eggs and young larvae.

Inoculum. Approximately 980×10^6 cells of *Bacillus pluton* were fed to the bees.

Date of inoculation. 25 June.

Results. 6, 7 and 12 days: No infected larvae seen. 18 days: Four infected larvae were removed from their cells. All had numbers of *B. pluton* cells in their intestines and variable numbers of *Bacterium eurydice* organisms. 21 days: Five infected larvae examined; all had large numbers of *Bacillus pluton* cells in their intestinal tracts, and some *Bacterium eurydice* cells. Further examinations 27 and 32 days after inoculation revealed large numbers of infected larvae, but the disease became less noticeable and finally almost disappeared toward the end of the brood-rearing season.

The results of the above experiment are interesting for they indicate that, as in the case of American foul brood (8), a relatively large inoculum of the causal organism is required to initiate the disease in healthy nuclei.

Modifications of European foul brood induced by spraying cultures of certain of the secondary invaders occurring in the disease over the developing brood of nuclei infected with Bacillus pluton

EXP. 3.

Nucleus. Stores; young larvae and sealed brood; eggs and young larvae (inoculated); eggs and stores.

Inoculum. 30 ml. of a suspension containing approximately 48×10^6 cells of *B. pluton* and 30×10^6 spores of *B. para-alvei* in M/5 phosphate buffer pH 7.2 were sprayed over the eggs and young larvae in the comb indicated.

Date of inoculation. 6 July.

Results. 4 days: Three of seven diseased larvae noticed in the inoculated comb were examined and all showed masses of *B. pluton* organisms and some *Bacterium eurydice* cells in their intestinal tracts. 10 days: Three of eight diseased larvae seen were examined, and all had the same appearance as noted above. 16 days: The queen had been killed (supersedure); every comb showed large numbers of slimy decomposing larvae which gave off a very foul odour. Ten of these were examined. The more recently infected larvae showed chiefly *Bacillus pluton* and *Bacterium eurydice* organisms, but the very slimy evil-smelling ones contained chiefly sporulating rods of *Bacillus para-alvei* and had a rather reddish tinge. A pure culture of *B. para-alvei* was isolated from one of them. 20 days: The bees had removed most of the dead larvae and scales as the nucleus was queenless. Three reddish, foul-smelling, sticky scales of dead larvae were found, and these contained *B. para-alvei* in almost pure culture.

EXP. 4.

Nucleus 2 (Exp. 1) was used, and had been affected with European foul brood for at least 23 days.

Inoculum. The centre comb containing plenty of eggs, young larvae and affected brood was sprayed with 25 ml. of an aqueous suspension containing approximately 31×10^6 *B. orpheus* spores.

Date of inoculation. 29 July.

Results. 7 days: A large number of moist, decomposing, light brown rather "buttery" larvae were present in all three brood combs. Four of these were examined; Three of them showed large numbers of vegetative cells and sporulating rods of *B. orpheus* in addition to *B. pluton* cells, and the fourth showed chiefly *B. alvei* rods and spores. A pure culture of *B. orpheus* was isolated from one of these larvae. 18 days:

Ten of a very large number of infected larvae seen were examined. Four of these were recently infected and showed chiefly *B. pluton* and *Bacterium eurydice*; three were light brown and rather "buttery" and contained numbers of *Bacillus orpheus* spores and vegetative cells; and three were brown, slimy and evil smelling and contained chiefly *B. alvei* vegetative cells and spores. The disease soon became less serious and by the 35th day following inoculation with *B. orpheus* no infected larvae could be seen. In the "buttery" larvae in which *B. orpheus* was principal auxiliary putrefactive agent the smell emitted was only slight in comparison with those in which *B. alvei* was the most prevalent secondary invader.

Exp. 5.

Nucleus 1 of Exp. 2 in which European foul brood had previously been initiated, was employed.

Inoculum. 20 ml. of a suspension containing approximately 27×10^{10} spores of a bacillus previously described ("Culture 1") (4) were sprayed over the developing brood in one comb which already contained some larvae affected with European foul brood.

Date of inoculation. 16 July.

Results. 6 days: A large number of affected larvae were seen. Three recently infected larvae examined showed chiefly *B. pluton* and *Bacterium eurydice* cells in their intestinal tracts. Two slimy, brown decomposing larvae contained large numbers of spores and vegetative cells of "Culture 1" in their tissues. A pure culture of this bacillus was isolated from one of them. 11 days: Large numbers of slimy, brown larvae: Five of these on examination showed quantities of spores and vegetative cells of "Culture 1". Subsequent examinations of the brood 20 and 28 days after inoculation revealed numbers of infected larvae, many of which contained chiefly spores and vegetative cells of the introduced bacillus. Larvae in which this organism constituted the main putrefactive agent gave off only a relatively slight odour.

Exp. 6.

Nucleus. Stores; young larvae and sealed brood; young larvae and sealed brood; eggs and young larvae (inoculated); eggs and sealed brood.

Inoculum. The eggs and young larvae in the comb indicated were sprayed with 20 ml. of a suspension containing approximately 78×10^7 *Bacillus pluton* organisms and 134×10^6 cells of *Streptococcus apis*.

Date of inoculation. 4 July.

Results. 3 days: Twelve affected larvae were noticed in the inoculated comb. Six of these were examined and all showed *Bacillus pluton* and *Streptococcus apis* cells and a variable number of small rod-shaped cells of *Bacterium eurydice*. Two of these had become yellow and of pasty consistency. 9 days: The disease had spread rapidly. Ten infected larvae including three drone larvae were examined. All of these contained *Bacillus pluton* and *Bacterium eurydice* cells and four of them showed large numbers of *Streptococcus apis* cells as well. 32 days: Large numbers of infected larvae were still present. Two of six examined showed numbers of *S. apis* cells in addition to *Bacillus pluton* and *Bacterium eurydice*. In this experiment the decomposing larvae, whether *Streptococcus apis* was present or absent, only gave off a slightly sour smell as of the "Sauerbrut" type of European foul brood.

The results of the above four experiments show that the appearance and odour of dead larvae in European foul brood is markedly affected by the presence of different

secondary invaders. Thus *Bacillus alvei*, which is the usual putrefactive agent, causes the larvae to become brown, slimy and evil smelling. *B. para-alvei* causes somewhat similar symptoms except that the larvae become rather red-brown in colour and emit a much more nauseating and more penetrating putrefactive odour. *B. orpheus* produces a somewhat "buttery" larva, rather light brown in colour, and only a slight odour results. The other bacillus studied caused a markedly slimy putrefaction of the affected grubs, which became dark brown in colour, but did not give off more than a slightly unpleasant odour. *Streptococcus apis* is associated with a type of disease which simulates the so-called "Sauerbrut".

Transmission of European foul brood by adult bees

Several attempts have been made to determine how European foul brood is carried by the bee. Bees usually remove larvae sick or dead of this disease by sucking the infected material from the cells; hence one would expect to find large numbers of bacteria in the intestinal tracts of nurse and house-cleaning bees in affected colonies. This has been found to be the case, for bees taken from combs containing large numbers of affected larvae have quantities of bacteria in their rectal ampullae. Usually, secondary invaders are most prominent, notably *Bacillus alvei* and *Bacterium eurydice*, but *Bacillus pluton* organisms are frequently present though, usually, they constitute a minority. The bacteria are invariably localized in the rectum, few or no bacteria being seen in the honey stomach, ventriculus or small intestine. It appeared possible that *B. pluton* might be carried in the pharyngeal or salivary glands of the bee. However, microscopical examinations of pharyngeal, mandibular or salivary glands of nurse and house-cleaning bees taken from colonies heavily infected with European foul brood have rarely shown any bacteria and never *B. pluton*. The following infection experiments show that *B. pluton* can exist in virulent form in the gut of the adult bee, and that crushed pharyngeal, mandibular and salivary glands of bees removed from affected colonies do not produce disease.

EXP. 7.

Nucleus. Eggs and young larvae; sealed brood; sealed brood; eggs and young larvae (inoculated); sealed brood and stores.

Inoculum. The rectal ampullae and their contents from six nurse or house-cleaning bees from a colony badly affected with European foul brood were triturated in 10 ml. of distilled water and the suspension was filtered through sterile cotton-wool in order to remove the coarser particles of cell debris. The resulting suspension which, on microscopical examination, showed *B. pluton* organisms together with quantities of vegetative cells of *Bacterium eurydice* and spores and vegetative cells of *Bacillus alvei*, was sprayed over the eggs and young larvae in the comb indicated.

Date of inoculation. 16 June.

Results. 3, 7 and 12 days: No diseased larvae seen. Some queen cells observed on the 12th day. 21 days: Had swarmed recently and was queenless; eggs still present. Fifteen affected larvae counted in one comb and a few noticed in each of the four remaining combs. Six of these showed *B. pluton* and the usual secondary invaders on examination. 25 days: Large numbers of infected larvae; Seventy-eight sick or dead grubs counted in the most badly affected comb. Five of these on examination showed the usual secondary invaders and *B. pluton*. Many of the larvae were brown, slimy and evil smelling and contained quantities of the spores and vegetative cells of *B. alvei*. Three queen cells which were present were examined, and a coiled larva affected with European foul brood was found in one of them. The nucleus was destroyed.

EXP. 8.

Nucleus. Young larvae and sealed brood; foundation; eggs, young larvae and sealed brood; eggs and young larvae (inoculated).

Inoculum. The pharyngeal, post cerebral, mandibular and thoracic salivary glands from ten nurse and house-cleaning bees from a colony badly affected with European foul brood were removed and triturated with 20 ml. of sterile brood filtrate (1:3), and the resulting suspension was sprayed over the comb indicated.

Date of inoculation. 10 June.

Results. Careful inspection of the brood 4, 7, 17, 23, 46 and 61 days after inoculation revealed no diseased larvae.

DISCUSSION

There can be little doubt that European foul brood is a single disease caused by an organism (or organisms) which has so far resisted all attempts at cultivation *in vitro*: the so-called *B. pluton* of White. This name, though the generic term is unsuitable, must be retained until the organism is cultivated. The species of lactic acid streptococci commonly designated *Streptococcus apis* do not always occur in larvae affected with European foul brood, and can be distinguished morphologically from *Bacillus pluton* which invariably occurs in such larvae. There is no reliable evidence which indicates that *B. pluton* is in any way related to these lactic acid streptococci.

Various modifications of European foul brood can be induced by introducing certain of the organisms occurring as secondary invaders in different forms of the disease (*B. para-alvei*, *B. orpheus*, *Streptococcus apis*, etc.) into colonies of bees which have been infected either simultaneously or previously with *Bacillus pluton*. The pronounced differences which have been observed with respect to smell, appearance and consistency of dead larvae in different cases of European foul brood, must be attributed to the presence of these auxiliary putrefactive bacteria. The fact that these secondary invaders are so variable in form and occurrence, makes it practically impossible to accept any theory that European foul brood is caused by a pleomorphic organism which can assume the form

of one or more of them. So far, no evidence has been obtained which suggests that *B. pluton* multiplies elsewhere than in the intestines, and probably in the peritrophic membranes, of very young larvae. The organism occurs in virulent form in the rectal ampullae of nurse and house-cleaning bees taken from colonies affected with European foul brood, but whether it exists for more than a brief time in this location is not known. Certainly it does not appear to multiply in the intestines of such bees, nor does it appear to be present in their pharyngeal, mandibular or salivary glands. *Pericystis apis*, the causal organism of Chalk brood, is frequently present in healthy colonies of bees and probably develops as soon as favourable conditions arise (2, 3), and it may be that the same is true of *Bacillus pluton*. The fact that European foul brood has been induced in healthy colonies of bees by the starved larva technique (5) supports this contention. Since the disease so frequently appears in weak colonies of bees in the spring it seems possible that *B. pluton* is indigenous to healthy colonies, and develops under suitable conditions. However, all attempts to induce European foul brood in healthy nuclei by artificially weakening them by removing sealed brood and young bees and introducing eggs and very young larvae from healthy colonies in their place, have failed. It is possible that success was not attained because the experiments were carried out somewhat after the peak of the brood-rearing season when the disease naturally decreases in severity. The results of experiments not recorded here have verified those previously made in which it was found that queens from colonies affected with European foul brood do not carry the disease when introduced into healthy nuclei.

Our knowledge regarding the cause of European foul brood cannot advance appreciably until *B. pluton* has been cultivated and, so far, all attempts to do this have failed. It is possible that the organism will grow on living tissues (tissue cultures), and this possibility must be investigated. The fact that attempts to grow the organism in the intestines of 3-4-day-old starved larvae have also failed suggests that it is a very strict parasite which will be extremely difficult to cultivate.

SUMMARY

Evidence has been submitted which supports the theory that European foul brood is a single disease caused by *Bacillus pluton* White.

The course of the disease can be modified by introducing cultures of certain secondary invading bacteria into colonies of bees infected with *B. pluton*.

A certain "mass inoculum" of *B. pluton* organisms is required to induce the disease in healthy colonies.

The causal organism is present in virulent form in the rectal ampullae of young bees in affected colonies, but does not appear to exist elsewhere in the bee or to multiply in its intestinal tract.

It appears as if *B. pluton* is a strict parasite which will only multiply in the intestines of young larvae.

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EXPLANATION OF PLATE XXXIX

(All photographs $\times 1200$)

- Fig. 1. *Bacillus pluton* in the intestine of a larva recently infected with European foul brood. This larva was apparently healthy and about 3 days old. The organisms are embedded in a fold of membrane: this is probably a portion of the peritrophic membrane.
- Fig. 2. *B. pluton* in the intestine of an apparently healthy larva about 3 days old. There appears to be a transition from the rather rod-shaped organisms, occurring chiefly in pairs and short chains, to the single, lanceolate-shaped cocci so characteristic of larvae in which the disease has become slightly more advanced.
- Fig. 3. *B. pluton* in apparently pure culture in the intestine of a larva about 4 days old. These lanceolate-shaped cocci rarely occur in pairs at this stage, and are usually single and grouped together as shown.
- Fig. 4. *B. pluton* in the intestine of a 4-day-old larva affected with European foul brood. A few small rods of *Bacterium eurydice* are beginning to appear. Note particularly the typical lanceolate-shaped cells which vary greatly in size.
- Fig. 5. *Bacillus pluton* and *Streptococcus apis* occurring together in a 4-day-old larva affected with European foul brood. The small group of lanceolate-shaped *Bacillus pluton* organisms can be distinguished from the rather spherical cells of *Streptococcus apis*. A few *Bacterium eurydice* rods are also present.
- Fig. 6. *Bacillus pluton* and *Streptococcus apis* occurring together in the intestine of a 4-day-old larva affected with European foul brood. Note the two types of bacterial cell which might readily be confused at first sight. The cells of *Bacillus pluton* are characteristically lanceolate-shaped: those of *Streptococcus apis* are almost spherical. A few small rods of *Bacterium eurydice* are also present.
- Note. All attempts to cultivate the cocci from the stomachs of the larvae used in making the preparations from which Figs. 1-4 were taken failed, while *Streptococcus apis* grew readily from the larvae employed in making the preparations for Figs. 1-6.

(Received 6 November 1936)



Fig. 1.



Fig. 2.

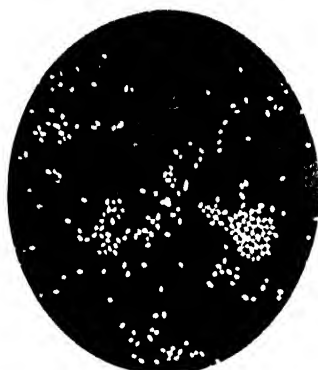


Fig. 3.



Fig. 4.

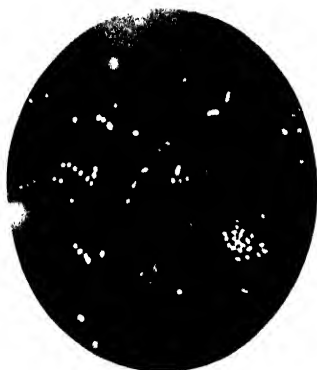


Fig. 5.

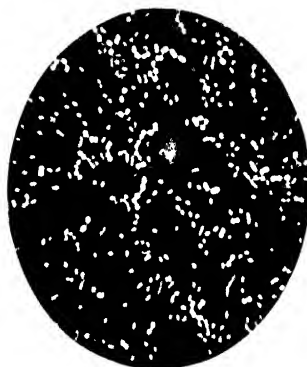


Fig. 6.

ADDLED BROOD OF BEES

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(With Plates XXVIII and XXIX)

THROUGHOUT the past few decades there have been numerous references in the German literature to "Eitaubheit" (addled egg disease), a disease in which fertile queen bees lay eggs which never develop into larvae. This disease has also been observed in England. The pertinent literature has been reviewed briefly by Buttel-Reepen (3) and Leuenberger (6), while Fyg, in recent articles (4, 5), has provided a fairly exhaustive résumé of the subject. Only the most important features of this anomaly will be discussed here, special reference being made to its apparent relationship with addled brood.

Many years ago it was shown that the queen is directly responsible for the condition known as Eitaubheit, and that the failure of the eggs to mature is certainly not due to a lack of attention by the nurse-bees of the colony. In most cases an embryo forms in such eggs but dies just prior to the normal time of hatching. All attempts to demonstrate any anatomical abnormality in queens laying addled eggs (Taube eier) have failed: their reproductive organs appear quite normal, and their spermatheca are full of active sperms. Eggs laid by defective queens in drone cells also fail to develop, and, since it is presumed that these have not been fertilized, it has been inferred that the queen is directly responsible for producing the defective eggs, and that the sperm plays no direct part. However, recent rather ingenious experiments by Fyg (5) have somewhat upset this hypothesis. He discovered that, after chilling for $16\frac{1}{2}$ hours at $0-2^{\circ}\text{C}$., a queen affected with Eitaubheit laid some eggs in drone cells which yielded normal drones. His results led him to suggest that inseminated eggs alone are addled, for chilling in this manner usually kills about half the sperm cells in a queen's spermatheca. It would seem possible that the fertilized eggs were addled and the unfertilized ones developed into drones. The true cause of Eitaubheit remains to be determined.

Writings referring to addled brood have been far less numerous than those dealing with addled eggs. In a book published in 1928 Leuenberger (6) described a case of Eitaubheit in which some of the eggs hatched,

but in which the few unsealed and sealed larvae developing from them died prior to reaching maturity. This appears to have been a mixed case of addled eggs and addled brood. It is impossible to say whether Leuenberger noticed this case prior to 1924 when Anderson⁽¹⁾, working in Scotland, first described addled brood; but it is fairly certain that both investigators worked independently. In the cases studied by Anderson the sealed brood was attacked, the larvae dying subsequent to pupation. The bodies of the dead larvae were pigmented, the chitin was hardened, the claws and setae were well developed and the wings still encased. Frequently the cappings over the dead brood became so sunken that, superficially, the disease might have been mistaken for American foul brood. The cases of addled brood described by Anderson were extremely severe, for frequently not a single bee emerged. In certain of these cases it was observed that a number of eggs failed to hatch, and this might be taken to indicate that such queens were suffering from Eitaubheit as well as addled brood. Anderson & Keith⁽²⁾ showed that the queen was directly responsible for producing addled brood by exchanging queens from healthy and affected colonies of bees. In one case investigated, though most of the brood failed to emerge, a few bees succeeded in doing so, but these never matured properly and were quite unable to fly, crawling about as if diseased.

Since the writer commenced studying brood diseases in 1934 a very large number of brood combs containing dead larvae have been examined, and in many of these the disease did not correspond with any of the well-recognized brood diseases. The appearance of the dead brood in such samples was described previously as "uncertain"⁽⁸⁾, and more recently, when it became apparent that the disease was closely similar to that described by Anderson, as addled brood⁽¹⁰⁾. Moreaux⁽⁷⁾, working in France, recently described a case of addled brood. He found that both drone and worker brood were affected, that many of the bees which did succeed in emerging were not properly developed and could not fly, and that the queen, on dissection, showed no abnormality.

DIAGNOSTIC FEATURES

In the writer's experience addled brood is a disease the seriousness of which varies greatly; a colony may only be slightly affected or markedly weakened, depending on the amount of sealed brood affected. The disease appears to be most pronounced at the peak of the brood-rearing season, but whether the actual proportion of affected to healthy pupae is greater

at this time is not known. Addled brood is certainly widespread in England, and it is not unusual for it to be mistaken for "foul brood" by inexperienced workers. The appearance of brood combs taken from affected colonies is shown in Pl. XXVIII, figs. 1 and 2, and a description of the dead brood follows.

The larvae normally die in the pupal or prepupal stage, that is to say, subsequent to sealing. Combs of sealed brood taken from affected stocks frequently exhibit a "pepper-box" appearance similar to that seen in cases of American foul brood. The affected larvae assume just such an appearance as might be expected if they had never been completely nourished, and had, therefore, failed to reach maturity and had consequently died and undergone autolysis. The dead prepupal larvae are soft and moist, and can only be removed intact from their cells with great difficulty. They are frequently greyish in colour, and as they dry rather moist, slightly sticky, brown scales form; but these are never as sticky and adherent as American foul brood scales. Normally there is no pronounced odour, though some of the decomposing larvae may give off a faint, not unpleasant, acid aroma. One of the most outstanding characteristics of the complaint is that pupae and bees almost ready to emerge from their cells die. In such cases the pupae are frequently small in size, the abdominal parts being especially restricted. The bees usually attempt to remove the dead pupae, removing the cappings and chewing the larval remains. Frequently dead pupae can be seen thrown out on the alighting board of the hive, or lying on the ground. Affected larvae are normally sterile, or almost sterile, bacteriologically, though occasionally small rod-shaped bacteria similar to White's *Bacterium eurydice* can be seen in the remains of dead pupae. The appearance of addled drone and worker pupae is shown in Pl. XXIX, figs. 3 and 4.

EXPERIMENTAL

Nuclei of hybrid bees, prepared as previously described⁽⁹⁾, were employed in the experiments. Queens were introduced by the usual travelling cage method, the nuclei being left for one or two days in a queenless condition prior to the introduction. The usual form of describing experiments and their results has been adopted⁽⁹⁾.

Experiment 1

Nucleus. Stores; eggs and young larvae; introduced comb containing very large numbers of autolysing addled pupae; eggs and young larvae; stores. Queen present.

Inoculum. One brood comb containing very large numbers of addled pupae which had been sent to the laboratory for purposes of diagnosis.

Date of inoculation. 28 June.

Results. 3 days: Practically all dead pupae had been removed from the introduced comb by the bees, and eggs were present in many of the cells. 11 days: Large areas of sealed brood on the introduced comb; a very few addled pupae still present. No affected larvae in adjacent combs. 15 days: Only one dead pupa seen in the introduced comb; healthy sealed brood in all other combs. Examinations 19, 27 and 35 days after the introduction of the comb containing the dead pupae revealed no disease. After 35 days two combs of sealed brood were removed and replaced with foundation to prevent overcrowding. Subsequent examinations after 68 and 78 days revealed no dead brood. The nucleus was wintered successfully and was examined on 28 April of the following year with the result that no addled pupae were seen.

Experiment 2

Nucleus. Stores; eggs and young larvae; introduced comb containing large numbers of dead pupae (addled brood); eggs and young larvae; stores. Weak in bees. Queen present.

Inoculum. A comb, similar to that used in Exp. 1, which had been sent to the laboratory.

Date of inoculation. 28 June.

Results. 3 days: A large number of dead pupae removed from the introduced comb, some still remaining. Eggs in many of the cells. 11 days: Some dead pupae still present in the introduced comb, none in the adjacent combs. 15 days: A few addled pupae still present in the introduced comb, but none in the adjacent comb. Weak in bees. 17 days: All the dead pupae removed from the introduced comb and no dead larvae seen in any brood comb. Subsequent examinations 19, 27, 35, 68 and 78 days after the introduction of the comb containing addled brood revealed no diseased larvae.

The results of these two experiments may be taken as evidence in support of the fact that addled brood, unlike the other brood diseases, is not contagious. Additional experiments which have not been reported here have confirmed this fact.

Experiment 3

Nucleus. Empty drawn comb; eggs and young larvae; eggs, young larvae and sealed brood; eggs and young larvae; stores.

Queen introduced. A queen which was producing addled brood in

small amounts, but not seriously, was obtained from an outside apiary in which the trouble had been prevalent for at least 2 years.

Date of introduction. 1 May.

Results. 2 *days*: Queen not liberated. 9 *days*: Eggs and young larvae. 38 *days*: Brood on five combs but no added pupae seen. 46 *days*: no added pupae observed. 53 *days*: Large numbers of added pupae noticed in all five combs. Two combs of sealed brood removed and one drawn "drone" comb and one comb containing eggs and very young larvae from a healthy nucleus were added. 63 *days*: Many young larvae and eggs in the drone comb. Healthy sealed brood in the comb of young larvae and eggs which had been added. The remaining three combs showed numbers of added pupae. 76 *days*: A very even comb of sealed drone brood present, and only one added pupa seen in it. No added pupae seen in the comb of eggs and young larvae which had been introduced though most of the brood had emerged and eggs and young larvae were present in the comb. Added pupae in the other two combs which contained brood. 79 *days*: Many drones emerged, or emerging, from the drone comb. This comb was removed, and was found to contain a number of added pupae, many in capped cells and a few in cells from which the bees had removed the cappings. Thirty-five added drone pupae were counted (see Pl. XXIX, fig. 3). 83 *days*: Breeding had slowed down but some added pupae were still present in the combs of sealed brood.

This experiment is of interest in that it shows that added brood is caused by the queen, may exist in a form mild enough not to visibly affect the strength of the colony, will persist through the brood-rearing season, and affects drone as well as worker brood. Eggs and young larvae from a healthy nucleus were raised normally by the bees of the nucleus in which the queen was producing added brood.

Experiment 4

Nucleus. Queenless. Made by taking three combs of brood (chiefly sealed) and bees from a healthy colony.

Queen introduced. A queen which was producing added brood was obtained from a colony in the home apiary, Rothamsted.

Date of introduction. 18 June.

Results. 4 *days*: Queen not liberated. 14 *days*: Eggs and young larvae in three combs, and a little sealed brood. No added brood seen. 29 *days*: A number of dead pupae in two combs of sealed brood. 40 *days*: A large number of added pupae in three combs of sealed brood. 48 and

52 days: Numerous addled pupae in all three combs of brood: the nucleus was rather weak in bees. Dequeened the nucleus, caging the queen with twelve young worker bees (used in Exp. 5). One comb was removed and photographed (Pl. XXVIII, fig. 1). **53 days:** Introduced a queen taken from a healthy nucleus with perfect sealed brood. **61 days:** Eggs and very young larvae in the centre comb, all the brood of the last queen either emerged or cleaned out by the bees. **68 days:** Sealed brood in centre comb, no addled pupae seen. Subsequent examinations 76 and 84 days after the commencement of the experiment revealed no addled brood.

Experiment 5

Nucleus. Sealed brood and eggs; eggs, young larvae and sealed brood; eggs and young larvae.

Queen introduced. The queen which had produced addled brood in Exp. 4.

Date of introduction. 9 August.

Results. **8 days:** Eggs and very young larvae from the introduced queen present. No addled brood seen. **15 days:** Sealed brood in two combs, four addled pupae seen. **22 days:** Abundant addled pupae in two combs and several of these partly removed by the bees. **30 days:** Breeding very slowly and united for winter with a queenless nucleus. Only a few affected pupae seen in one comb.

These two experiments show that addled brood is produced by an affected queen throughout the brood-rearing season even when she is transferred to two different nuclei. Requeening an affected nucleus with a healthy queen causes the disease to disappear.

Experiment 6

Nucleus. Queenless; three combs with a little sealed brood and bees.

Queen introduced. A queen which had headed a colony of bees from which a sample of addled brood (a fairly severe case in which most of the sealed brood was affected) had been sent to the laboratory for diagnosis.

Date of introduction. 1 July.

Results. **6 days:** Eggs present. **15 days:** One comb of sealed brood containing very large numbers of addled pupae, many of which had been chewed and partly removed by the bees. **21 days:** Addled brood very marked, a large number of affected pupae in all combs containing sealed brood. **35 days:** Breeding more slowly and addled brood not so noticeable as previously. **40 days:** Addled brood still present but less marked.

One comb removed and photographed (Pl. XXVIII, fig. 2). Pupae affected with addled brood were carefully removed from this comb and photographed (Pl. XXIX, fig. 4). 49 and 63 days: Breeding slowly, only a few affected pupae seen.

Experiment 7

Nucleus a. Eggs and sealed brood; eggs and sealed brood; sealed brood; stores. Addled brood present in fairly large amount. Queen mated in 1936 and addled brood was noticed in the first sealed brood reared from her eggs.

Nucleus b. Eggs, young larvae and sealed brood; sealed brood; young larvae and sealed brood. No addled brood present and queen apparently normal.

The queens from both nuclei were removed on 15 July and, after 24 hours, were interchanged.

Results. Nucleus a. 12 days: Only a few addled pupae remained, eggs and young larvae from the introduced queen present. 20 days: No diseased larvae seen; eggs, young larvae and sealed brood in abundance. Subsequent examinations after 32, 46 and 51 days revealed no addled brood.

Nucleus b. 20 days: Breeding slowly, some addled pupae present. 32 days: Only a few addled pupae seen. 47 days: One comb contained a large number of affected pupae, and another comb contained a few. 55 days: Only two addled pupae seen; united for the winter with a queenless nucleus.

The results of these experiments may be looked upon as additional evidence in support of the fact that addled brood is produced by a defective queen, and that recently mated queens will produce the disease.

DISCUSSION

Though the superficial cause of addled brood is a defective queen, the fundamental cause remains to be determined. The complaint seems similar to addled egg disease, but whether the underlying cause of both diseases is the same is not known. From the fact that both anomalies have been observed simultaneously in the same colonies of bees it might be inferred that there is some relationship. Addled brood may be hereditary, the queen possessing some lethal factor, or she may suffer from some infectious disease or from some pathological abnormality. These and other questions relating to this disease remain to be determined through practical experiments.

SUMMARY

Addled brood is produced by defective queen bees and is not of a contagious nature as are the other common brood diseases.

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EXPLANATION OF PLATES XXVIII AND XXIX

(Photographs by V. Stansfield)

PLATE XXVIII

Fig. 1. Comb showing addled brood: the cappings over the dead pupae have been removed by the bees in most cases and they can readily be seen. Very young larvae can be seen in the open cells. This comb was obtained from the nucleus used in Exp. 4 on 8 August, and at this time usually there are much fewer dead pupae than at the peak of the brood-rearing season.

Fig. 2. A comb containing addled brood taken from the nucleus used in Exp. 6 on 10 August.

PLATE XXIX

Fig. 3. Addled drone pupae from a drone comb used in Exp. 3.

Fig. 4. Addled worker pupae from the comb shown in Fig. 2. Note the small shrivelled bees and soft autolysing pupae (compare with Fig. 5).

Fig. 5. Healthy young adult bees recently emerged and advanced pupae from a healthy stock of bees.

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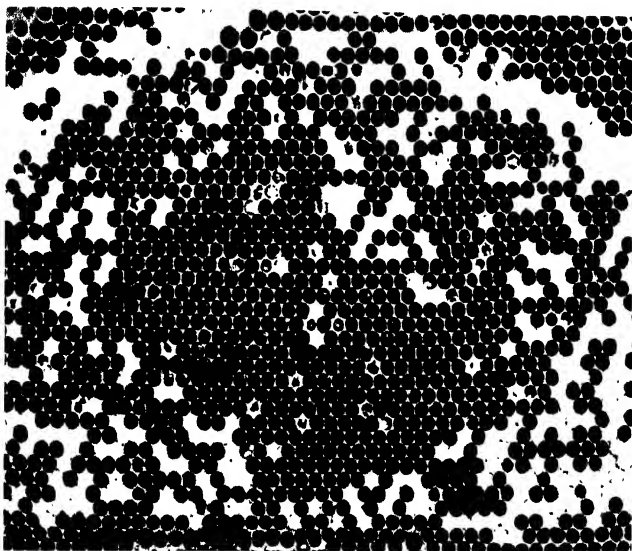


Fig. 1

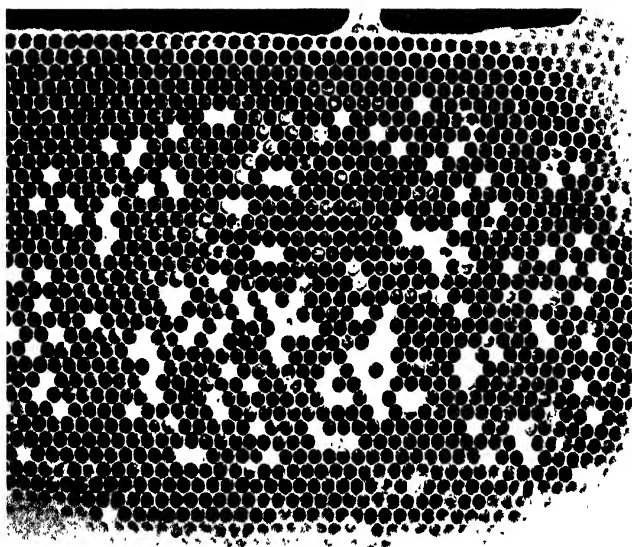


Fig. 2

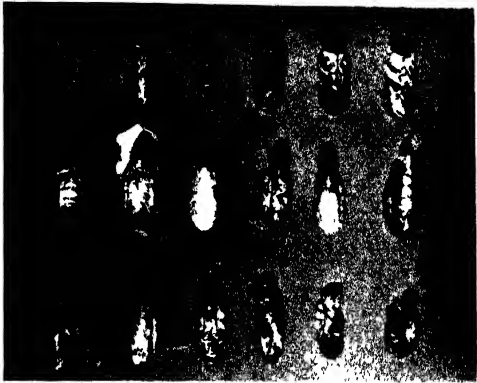


Fig. 3



Fig. 4

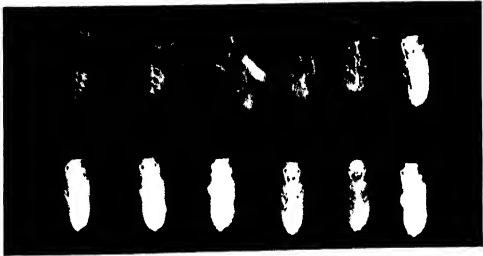


Fig. 5

STUDIES ON AMERICAN FOUL BROOD OF BEES

I. THE RELATIVE PATHOGENICITY OF VEGETATIVE CELLS AND ENDOSPORES OF *BACILLUS LARVAE* FOR THE BROOD OF THE BEE

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THE discovery that American foul brood was a distinct disease caused by a spore-forming bacillus was first made by White⁽¹²⁾, though Maassen⁽⁴⁾, working independently, came to the same conclusion shortly afterwards. White termed the causal organism *Bacillus larvae*, and this designation was subsequently accepted by Maassen. Maassen⁽⁴⁾ produced American foul brood in colonies of bees by feeding them pure cultures of *B. larvae*, but it is not clear whether his cultures contained only the vegetative cells of this organism or the spores as well. Later⁽⁵⁾ he found that *B. larvae* rapidly loses virulence and ability to form spores when cultivated for some time on laboratory media. White⁽¹³⁾ produced American foul brood by feeding spore-containing brood filtrate or egg-agar cultures of *B. larvae* to the bees of healthy colonies, but he does not record that disease was produced by feeding the vegetative cells of this organism alone. He also found that direct inoculation of larvae with cultures of the causal organism rarely produced the disease. In France, Toumanoff⁽¹¹⁾ failed in his attempts to produce American foul brood by individual feeding of the young larvae of a healthy colony of bees with large numbers of the vegetative cells of *B. larvae*, and Chalmers⁽¹⁾ also experienced difficulty in infecting colonies with pure cultures of this organism. Sturtevant⁽⁷⁾ showed that, normally, a healthy colony of bees will not contract American foul brood unless the bees are fed at least 50 million spores of *B. larvae*, obtained from the scales of dead larvae, in 1 litre of sugar syrup. In his experiments individual larvae did not contract the disease unless they were fed 10 million or more spores of this organism in 0.01 c.c. of syrup.

When considered collectively, the above results indicate that the vegetative cells of *B. larvae* do not cause American foul brood readily, if at all; that the spores of this species are effective in producing the disease only when introduced in large numbers into healthy colonies; that the

direct feeding of advanced larvae is not a satisfactory method of inducing the disease; and that the causal organism attenuates with respect to virulence following cultivation on laboratory media. None of the above-mentioned investigators described the optimum conditions for spore formation by *B. larvae in vitro*, though White⁽¹³⁾ mentions that spores were formed on his brood filtrate and egg-agar media. The experiments to be described were undertaken with the object of devising a medium which would prove suitable for obtaining large numbers of the spores of *B. larvae*, and to determine the relative infective power of vegetative cells and spores of this species for the brood of the bee.

EXPERIMENTAL

A medium for obtaining vegetative cells and spores of B. larvae

Early attempts to infect healthy nuclei with vegetative cells of *B. larvae* by feeding the brood directly were unsuccessful⁽⁹⁾, and, therefore, energy was directed toward finding a medium upon which this species would sporulate readily. Since it is known that *B. larvae* is a fastidious organism and will only grow on rather complicated media^(1, 3, 4, 13) and that, normally, spore formation by aerobic bacilli is enhanced by a reduction in the concentration of available nutrients⁽⁸⁾, the problem was a somewhat difficult one. A number of media upon which this organism grows readily (egg-yeast-peptone agar⁽⁷⁾, beef-digest agar⁽²⁾, beef-infusion agar, and others; with and without the addition of brood filtrate (*v. infra*)) were studied, but spores were rarely, if ever, formed on these. It was found that *B. larvae* grows well on agar containing only a small amount of sterile brood filtrate and that, on this medium, it sporulates slightly. However, brood filtrate contains sugars which this organism ferments with the formation of acid, and it is known that an acid medium normally inhibits sporulation. By neutralizing the acid formed by adding a buffer salt to the medium, a substrate favouring spore formation by *B. larvae* was finally evolved. Phosphate buffer was found to be a more suitable buffer salt than calcium carbonate with the medium used. The following medium has so far proved most satisfactory for obtaining large numbers of the spores of *B. larvae*: 1.5 per cent agar, 10 ml.; brood filtrate (1 : 5), 1 ml.; M/1 phosphate buffer, pH 7.2, 1 ml.; tubed in 6 by 1 in. test-tubes. The phosphate buffer and brood filtrate are added to the liquid agar (45–50° C.) prior to sloping. Brood filtrate is prepared by triturating unsealed drone or worker larvae in a mortar, adding 4 parts of water to 1 of crushed brood, and autolysing for some days over chloroform. On standing, the solids settle and the supernatant liquid is first filtered

through filter paper and, finally, through a Seitz filter, in order to sterilize it.

Experience has shown that *B. larvae* rapidly loses the ability to form spores when transferred on laboratory media, even when the above substrate is used, and in this the findings of Maassen have been verified. The following has proved to be the most effective method of obtaining spores from pure cultures of *B. larvae* for infection experiments. It is to be noted that throughout this work an incubation temperature of 35° C. has been employed. Portions of the scales or, preferably, the ropy remains of larvae dead of American foul brood, are streaked over the surface of beef-digest-brood-filtrate agar⁽¹⁰⁾ slopes, aseptic precautions being observed. Abundant growth of *B. larvae* in apparently pure culture normally occurs when such cultures are incubated for 48 hours. A fairly large inoculum must be employed to effect growth with all recently isolated cultures of this species⁽⁶⁾. Portions of the resulting growth are thoroughly emulsified in beef-digest broth, dilutions are made employing the same medium, and Petri plates are poured using beef-digest-brood-filtrate agar. Colonies usually appear on these plates after 2-3 days' incubation, and several slopes of the same medium are inoculated from a single colony. These cultures are incubated for 24-48 hours and portions of the resulting growth are streaked over the surface of a large number of tubes containing the above described buffered brood filtrate agar. When vegetative cells of *B. larvae* are required these cultures are incubated for not longer than 48 hours, while an incubation period of 1 week is employed when spores are required. Large numbers of spores are formed in such recently isolated cultures, though by no means all the vegetative cells sporulate and quite a large proportion die and autolyse without doing so. The vegetative cells are washed from the medium with beef-digest broth, and the spores with *M*/5 phosphate buffer, pH 7.2. It has been found that most of the clumps of vegetative cells can be broken up by shaking, and that the same procedure is effective in the case of clumps of spores providing the shaking is carried out in the phosphate buffer. The cell suspensions are concentrated by centrifuging and resuspending the precipitate in broth or buffer solution. The number of spores or vegetative cells in a given suspension is estimated by use of a Thoma haemocytometer slide, it being found that the addition of a few drops of a 5 per cent sodium hydroxide solution facilitates the breaking up of small clumps of cells prior to counting. The addition of a few drops of a strong solution of methylene blue to the suspension also makes counting easier.

In the experiments to be described, the presence of American foul brood in a given nucleus was diagnosed by the examination of a fairly large number of affected larvae in order to identify the spores of *B. larvae*, and by the isolation of a pure culture of this organism from one affected larva. The morphological, cultural and biochemical characteristics of this species have been described frequently (3, 4, 6, 13).

The production of American foul brood in healthy nuclei

In the following experiments the preparation of nuclei, the technique of inoculation and the record of experimental results are similar to those previously employed (10). A De Vilbiss No. 15 glass (sterilizable) atomizer was employed to spray developing brood in certain of the following experiments.

Experiment 1.

Nucleus. Stores; sealed brood; eggs and young larvae (inoculated); eggs, young larvae and sealed brood; eggs, young larvae and sealed brood.

Inoculum. Approximately 8.4×10^{10} vegetative cells of *B. larvae* in 20 ml. of beef-digest broth were sprayed over the eggs and young larvae in the comb indicated.

Date of inoculation. 10 June.

Results. No diseased larvae were seen on careful inspection 6, 10, 14 or 28 days after inoculation. 34 days: The queen had been killed (supercedure). No diseased larvae were seen and the experiment was concluded.

Experiment 2.

Nucleus. Eggs and young larvae (inoculated); young larvae and sealed brood; eggs and young larvae.

Inoculum. Approximately 17.2×10^{10} vegetative cells of *B. larvae* in 25 ml. of beef-digest broth were sprayed over the eggs and young larvae in the comb indicated.

Date of inoculation. 11 July.

Results. Some of the sprayed larvae removed, no diseased larvae seen. Subsequent examinations 11, 15, 25, 41 and 51 days after inoculation revealed no diseased larvae and the experiment was concluded.

In the above experiments American foul brood was not initiated by spraying vegetative cells of *B. larvae*, obtained from recently isolated cultures, over developing brood. The results are in harmony with those previously obtained when it was found that this disease was not induced

when individual larvae were fed large doses of the vegetative cells of this organism(9).

Experiment 3.

Nuclei. No. 1. Stores; eggs, young larvae and sealed brood; sealed brood, eggs and young larvae.

No. 2. Eggs and sealed brood; eggs, young larvae and sealed brood; eggs and stores.

No. 3. Stores and sealed brood; eggs and young larvae; eggs and young larvae.

No. 4. Young larvae and sealed brood; eggs and young larvae; eggs and sealed brood.

Inocula. The bees of each nucleus were fed varying doses of the spores of *B. larvae* in 100 ml. of syrup(10) as follows:

Nucleus 1.	62 × 10 ⁶ spores
„ 2.	620 × 10 ⁶ „
„ 3.	6,200 × 10 ⁶ „
„ 4.	62,000 × 10 ⁶ „

Date of inoculation. 7 August.

Results. 10 and 14 days: No diseased larvae seen. 20 days: Nuclei 1 and 2 showed no diseased larvae. Nucleus 3 showed seven slimy decomposing larvae, two of which on examination were found to contain large numbers of *B. larvae* spores. A pure culture of this organism was isolated from one of these. Nucleus 4 showed thirteen ropy larvae, two of which on examination were found to contain large numbers of *B. larvae* spores. A pure culture of this organism was isolated from one of these. 24 days: Nuclei 1 and 2 showed no diseased larvae. Nucleus 3 had three ropy larvae full of spores of *B. larvae*. Nucleus 4 had two ropy larvae full of *B. larvae* spores. 32 days: No diseased larvae seen in Nuclei 1 and 2. Nuclei 3 and 4 contained one and two ropy larvae respectively, all three being full of spores of *B. larvae*. All four nuclei were breeding slowly, so concluded the experiment.

Experiment 4.

Nuclei. No. 1. Eggs and young larvae (inoculated); young larvae and sealed brood; eggs and stores.

No. 2. Eggs, young larvae and sealed brood; eggs, young larvae and sealed brood (inoculated); stores.

No. 3. Sealed brood; eggs and young larvae (inoculated); drawn comb; young larvae and sealed brood.

No. 4. Sealed brood and young larvae; eggs and young larvae (inoculated); young larvae and sealed brood.

Inocula. The combs of developing brood indicated in each of the above four nuclei were inoculated by spraying approximately the following numbers of spores suspended in 20 ml. of water (the spores employed being derived from exactly the same source as those used in Exp. 3):

Nucleus 1.	6.2×10^6	spores
„ 2.	62×10^6	„
„ 3.	620×10^6	„
„ 4.	6200×10^6	„

Date of inoculation. 7 August.

Results. 10 days: No diseased larvae seen in any of the four nuclei though in all cases some of the sprayed brood had been removed by the bees. 14 days: Nuclei 1 and 2 showed no diseased larvae. Nucleus 3 had three ropy larvae full of *B. larvae* spores and a pure culture of this organism was isolated from one of these. Nucleus 4 showed ten ropy larvae, six of which were examined and found to be full of spores of *B. larvae*. This organism was isolated in pure culture from one of these. 20 days: Nucleus 1 showed no diseased larvae. Nucleus 2 showed seven ropy larvae all of which contained spores of *B. larvae* in large numbers. This organism was isolated in pure culture from one of these. Nucleus 3 had twenty-three ropy larvae and Nucleus 4 a much greater number, the disease having spread rapidly. 25 days: Nucleus 1 breeding quite well but no diseased larvae seen. Nuclei 2, 3 and 4 showed two, nine and twelve infected larvae respectively. In Nuclei 2 and 3 breeding was very slow. The experiment was concluded.

In these two experiments American foul brood was initiated in healthy nuclei by feeding 6200×10^6 or $62,000 \times 10^6$ spores of *B. larvae*, obtained from pure cultures, to the bees. Smaller inocula did not produce the disease. On the other hand when spores from exactly the same source were sprayed over the developing brood of healthy nuclei 62×10^6 spores caused disease, but 6.2×10^6 spores did not.

DISCUSSION

The above experiments constitute the first attempt to compare the relative infective power of vegetative cells and spores of *B. larvae* for the brood of the bee. In these experiments the vegetative cells and spores of the organism were prepared under exactly similar conditions except for the length of time of incubation, and it was found that very large

doses of vegetative cells sprayed over developing brood produced no disease in inoculated nuclei, while much smaller doses of the spores of this organism soon caused American foul brood. As yet, no really satisfactory explanation of these results has been found. However, it seems probable that the vegetative cells are rapidly destroyed by the digestive processes of the larva, while the more resistant endospores survive the bactericidal mechanisms of the larval gut until such time as conditions favour their multiplication. That very young larvae are more susceptible to infection than older unsealed larvae is suggested by the fact that spraying them with a dilute spore suspension soon initiates American foul brood, while very large doses of spores are necessary to cause disease when fed directly to more advanced larvae (7). Perhaps the spores become enveloped in the peritrophic membrane of young larvae, while in older larvae they are destroyed by digestive enzymes before they reach a locality which favours their development. Certainly normally fed, and starved, larvae destroy such organisms as "*Streptococcus apis*" and spores of *Bacillus alvei* (10). If a method of maintaining *B. larvae* in the vegetative stage could be evolved then the control of American foul brood might be simplified. Unfortunately, the possibility of doing this seems rather remote at present.

In the above experiments it was found that a relatively large inoculum of the spores of *B. larvae* was required to produce American foul brood in a healthy nucleus; and that a much smaller inoculum was effective in producing this disease when the developing brood of the nucleus was sprayed directly with the spores than when they were fed to the bees in syrup. Sturtevant (7) found that a colony of bees would not develop American foul brood unless the bees were fed at least 50 million spores of *B. larvae*, obtained from scales of dead larvae, in 1 l. of sugar syrup; while, in the above experiments, the limiting infective dose was approximately 6200 million spores obtained from pure cultures of the organism. This apparent discrepancy may well be due to the fact that *B. larvae* rapidly attenuates with respect to virulence when cultivated on laboratory media.

SUMMARY

Vegetative cells of *Bacillus larvae* have not produced American foul brood in healthy nuclei even when a dose almost 3000 times greater than an inoculum of spores of this organism capable of causing the disease has been sprayed over the developing brood.

A very much smaller inoculum of the spores of *B. larvae* is effective in producing American foul brood when the developing larvae of healthy

nuclei are sprayed directly with them than when they are fed in syrup to the bees.

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A PHYSICAL TEST FOR LING HONEY

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THE judging of honey at shows is an art that requires considerable experience and is always open to a certain amount of criticism. No two judges are certain to give the same awards. The same judge may make different decisions on different occasions. Even when using the score card system, it cannot be said that the merits of different honeys can be numerically expressed. In other words, so-called "organoleptic tests" are uncertain, and it is impossible to convey with any accuracy the grounds on which honeys of a series under consideration are placed in a given order.

Tea and wine tasters are, no doubt, of great assistance to the firms that employ their services, but the excise department and the analyst require specific gravity tests and chemical reactions that do not depend on a man's opinion. Definite data are being obtained, on which such commodities as malting barley and flour can be evaluated on grounds more convincing and reliable than the pronouncements of an expert, however long his experience may have been.

It seems desirable that attempts should be made to express the quality of honey similarly in terms of analysis or physical properties that may be determined precisely, by means of tests that are capable of repetition, and as far as possible to exclude the personal factor of judgment, however skilled. The inclusion of honey in the National Mark scheme meant that honey so packed had to conform to certain standards of clarity, colour, water content (conversely, total solids) and sucrose (cane sugar) content. A limit was placed on the percentage of water allowed, on the grounds that fermentation is apt to occur if it exceeds a certain amount. This was fixed on the safe side, as sales of honey are much injured if honey that is fermented or otherwise below standard is offered for sale.

The amount of water present may be determined from the density of the honey. There has been much confusion among beekeepers in the past over the term density: it has often been treated as synonymous with viscosity.

Density or specific gravity can be measured by weighing

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a sample of known volume, or by the use of a hydrometer which floats high or low according to the density of the honey or other liquid. The hydrometer is a familiar method of testing the acid in an accumulator. The specific gravity bead, which is recommended for the use of honey packers, works on this principle. This is the commonest way of arriving at the water content of honey. Density varies slightly with temperature but allowance can be made for this.

The *viscosity* of a liquid, on the other hand, is a measure of its flow properties, and is not necessarily connected with its density. Heavy oils, for example, flow slowly and are of high viscosity, but of lower specific gravity than water, on which they would float. The viscosity of honey, though a distinct property, is related to the specific gravity because honey is not a simple liquid like an oil, but a solution of sugars in water. The viscosity of honey varies considerably with the temperature.

When the test of density was applied to heather honeys, many samples were found to have too high a water content to be included within the limits of the National Mark scheme. As it seemed that this excess of moisture did not in all cases allow the honey to ferment, these honeys were examined to see if they possessed any special property that might reasonably excuse departure from this standard. The jelly-like nature of ling honey has long been known in connexion with the production of bottled heather honey. Because of this property it is not possible to use the centrifugal extractor in the ordinary way. The usual method is to press the combs and so destroy them. In recent years the Dutch have to some extent used a machine that, by dropping loose steel rods into the cells, breaks up the jelly structure and allows some air to enter. If combs are put into the ordinary extractor within twenty minutes or so of being so treated, it is then possible to remove the honey by centrifugal force in the usual way.

The Flow Properties of Heather Honey. It has been shown¹ that honeys from a great variety of sources, not including heather, behave almost as "true fluids." This means that if, for example, such honeys are forced through narrow tubes under pressure, they flow at a rate proportional to the pressure applied, and also that no amount of stirring,

¹ Paine, Gertler and Lothrop: Colloid Constituents of Honey, *Ind. & Eng. Chem.*, 26, 1934, 73-81.

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either caused by the flowing process itself or by deliberate mixing, will affect the ease of flow.

Now ling (*Calluna*) honey is quite unlike a "true fluid."¹ If it is forced through a tube, or if a small ball is allowed to fall through it, the rate of flow is not proportional to the pressure applied. For example, when the pressure is doubled it will yield at a rate which is far more than doubled. Moreover, newly-stirred honey, whether the stirring is deliberate or produced by flowing in some kind of test, flows much more readily than honey that has been left to stand for some time. Both these properties are well-known with other materials. The former has been called "structural viscosity" and the latter "thixotropy." With ling honey, the two are clearly connected. There is good reason to suppose that in the honey, the sugar molecules tend to arrange themselves into a sort of structure rather like scaffolding around a building. When the honey is stirred, or caused to flow, this structure is temporarily destroyed, and only re-establishes itself gradually, and it is natural that the bigger the pressure used, the more complete is the temporary destruction of the structure.

A convenient method of observing how far any particular honey shows such a capacity to form a structure has been described by Scott-Blair.² A $\frac{1}{4}$ -in. steel ball-bearing is dropped through a sample that has been allowed to stand for some time, then the sample is stirred thoroughly, and another ball is dropped. With ordinary honey the two balls will fall at the same rate, but with heather honey, the ball in the unstirred honey will fall many times more slowly than that in the stirred. If, for example, in a given sample the ball falls ten times as slowly, this honey is said to have a "thixotropy ratio" of ten. Thixotropy ratios depend on such things as temperature, size of ball used in test, etc., but under fairly standard conditions, they give a good indication of how much "structure" a honey is capable of building up. Some of the water in the honey is needed for this purpose, and it is known that water so utilized has properties different from those of ordinary water. Since honeys that show marked structure can presumably hold more water without fermenting than can structureless honeys, it is reasonable to suppose

¹ De Boer and Kniphorst: Thixotropie van Heidenhonig, *Chem. Weekblad*, 29, 1932, 526-534.

² Scott Blair: The Thixotropy of Heather Honey, *Jour. Phys. Chem.*, 39, 1935, 213-219.

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that water which is bound into the structure is not available for the use of ferments. Some ling honeys produce such a rigid structure that after some hours standing they set into solid jellies. Such honeys often give thixotropy ratios of as much as several thousand, i.e., a steel ball falls through them some thousands of times faster after vigorous stirring, than it did before.

It is important to inquire what plants give thixotropic honey, and whether the conditions of growth of the plant (soil, climate, etc.) affect the extent of the thixotropy or structural viscosity. For this purpose, samples of honey are required from sources that the beekeeper can guarantee.

There has been designed a test that necessitates only a few drops of honey, an obvious advantage, since a beekeeper could be sure of the source of a small corner of a comb, when he could not guarantee that the whole comb had been laid down from the same plant.

Although very small ball bearings are obtainable, there are serious technical difficulties in using the falling ball methods for such minute samples, and a flow method has been devised, this, however, involving the use of somewhat complex apparatus, so that it is intended for the research laboratory. In the meantime the authors would be grateful for samples of heather (ling) honey from any beekeepers who can guarantee a pure ling source, and supply some information as to the soil and climatic conditions where the plant grew. About a thimblefull of honey is ample.

The above methods are not the only possible ways of studying these phenomena. A great many methods are possible, but these have been chosen as the most suitable for this particular problem.

The Specific Nature of the Plant. It is a very curious fact that whereas most honeys derived from ling (*Calluna vulgaris*) are highly thixotropic, those derived from heathers such as *Erica cinerea* and *Erica tetralix* are not. Although Paine, Gertler and Lothrop (*loc. cit.*) were not looking for thixotropy among the honeys from so many sources which they examined, they could not have failed to note and comment on such a phenomenon had it occurred to any marked degree. With the possible exception of buckwheat, no plants that have been examined, give thixotropic honeys except ling (*Calluna vulgaris*), and the New Zealand plant manuka (*Leptospermum*

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scoparium). There may, of course, be others, but even so the phenomenon is exceedingly rare. Thixotropy must be due to the presence of some chemical substance that reaches the honey through the plant. Its presence clearly depends on the nature of the plant, and possibly, also on the chemical composition of the soil in which the plant grows. This matter is being further investigated at Rothamsted.

Details of the Thixotropy Test. Since the thixotropy ratio obtained for a honey depends to some extent on the exact way in which the test is done, it is as well that the following method should be followed as closely as possible.

The honey is poured into a tube known as a "100-cc. Nessler tube" so as to fill the tube right to the top, and left to stand overnight. (The tubes may be obtained from manufacturers of chemical apparatus at a cost of about 2s.) Granulated honey must not be used for the test. The tubes are marked with two lines about $2\frac{1}{4}$ in. apart. For the first test the $\frac{1}{4}$ -in. ball bearing is laid on the surface of the honey in the middle of the tube* and the times at which the centre of the ball passes the two lines are recorded. The second hand of an ordinary watch is adequate for this purpose, though if a stop watch is available, it is somewhat more convenient.

For the second test a metal disk perforated with holes is mounted on a metal rod so that it just slides freely in the glass cylinder.†

The honey is stirred by pushing this stirrer to the bottom of the cylinder and withdrawing it; this is done twice. *Immediately* after this, the ball-bearing is dropped and the time taken in passing the two lines is recorded as before.

The "thixotropy ratio" is simply obtained by dividing the time taken for the ball to fall between the marks in the first test by that taken in the second test. In order to see the ball in honeys that are opaque owing to air bubbles, it is best to stand the tube on a window-sill with the daylight for background, and to stand back slightly from the tube when making the observations. A good light is essential.

Since writing this article, the authors have read with great interest a paper by Mr. J. Pryce Jones (*Bee World*, August 1936), in which the thixotropy of honey is discussed.

* If the honey is opaque with air bubbles, the ball, to be visible, may have to be placed slightly to one side, but it should never be allowed to fall near the wall of the tube.

† Arrangements are being made to have these placed on sale.

Ein Mikroviskosimeter für Nicht-Newton'sche Flüssigkeiten.

Von G.W. Scott Blair¹⁾.

(Eingegangen am 23. Oktober 1936.)

(Physikalische Abteilung der Rothamsted Experimental Station, Harpenden.)

Einleitung.

Bei Untersuchungen über die Viskosität von Honig war die Feststellung notwendig, in welchem Maße thixotrope Honigsorten vom Hagen-Poiseuille'schen Gesetz abweichen. Diese Messungen wurden mit Hilfe eines Kugelviskosimeters durchgeführt²⁾. Wenn das Versuchsmaterial vollkommen klar ist und in genügend großen Mengen zur Verfügung steht, dann ist unserer Ansicht nach das Kugelviskosimeter immer noch das geeignetste Instrument zur Untersuchung thixotroper Systeme. Bisweilen jedoch stößt die Beschaffung größerer Mengen des Versuchsmaterials auf Schwierigkeiten, und außerdem muß die Meßmethode weitgehend modifiziert werden, um auch auf undurchsichtiges Versuchsmaterial anwendbar zu sein. Der hier beschriebene Apparat gehört zur Gruppe der Kapillarkviskosimeter. Mit nur 0,5 ccm Versuchsmaterial kann man die absolute Viskosität über weite Viskositätsgebiete mit zufriedenstellender Genauigkeit messen und auch die Änderung der Viskosität bei allmählichem Anstieg von Druck und Scherung bestimmen.

Bei dem Apparat von Bingham und Murray³⁾ wird ein leeres Kapillarrohr mittels komprimierter Luft bei konstantem Druck allmählich mit dem Versuchsmaterial gefüllt. Die Viskosität ergibt sich aus dem Verhältnis der Füllgeschwindigkeit zum Druck, die beide von der Länge der Flüssigkeitssäule abhängig sind. Für thixotropes Versuchsmaterial, bei dem die Viskosität mit wachsender Scherung und steigender Spannung kleiner wird, ist die Methode unbrauchbar, da der Druck in demselben Maße fällt, wie die Kapillare sich füllt, so daß also die durch die Thixotropie hervorgerufene Viskositätserniedrigung durch die Erhöhung infolge der fallenden Spannung teilweise kompensiert wird. Man findet daher nur minimale Abweichungen vom Hagen-Poiseuille'schen Gesetz. Wird die Kapillare jedoch zuerst mit der zu untersuchenden Flüssigkeit gefüllt und dann erst nach einer genügend langen Ruheperiode durch komprimierte Luft bei konstantem Druck entleert, dann ergibt der steigende Druck zusammen mit der

Scherung maximale Abweichungen vom Hagen-Poiseuille'schen Gesetz.

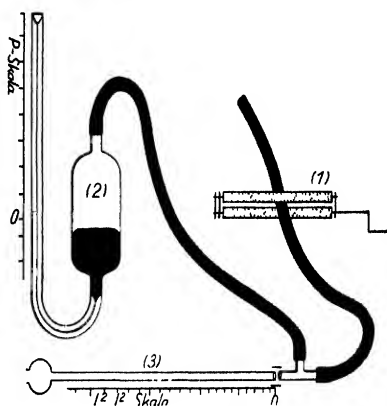


Fig. 1

Die Berechnung der Viskosität aus der Leerungsgeschwindigkeit der Kapillare.

Nehmen wir an, daß an den Enden einer langen, engen Röhre von der Länge L und dem Radius R , die mit dem Versuchsmaterial gefüllt ist, eine Druckdifferenz P (cm Hg) besteht. Ein Teil des Druckes P_1 ist erforderlich, um die Oberflächenspannung an den Menisken auf beiden Enden der Flüssigkeitssäule zu überwinden. Der Bruchteil P_2 dagegen verursacht die viskose Strömung.

Wenn das Versuchsmaterial eine ideale Flüssigkeit ist, dann können wir schreiben:

$$13,55 \text{ g } P_1 = 2 \times 2 \sigma / R = 4 \sigma / R \quad (1)^*$$

$$13,55 \text{ g } P_2 = v \times 8 l \eta / R^2 \quad (2)$$

wobei σ die Oberflächenspannung bedeutet, v die Bewegungsgeschwindigkeit des Meniskus, l die Länge der Flüssigkeitssäule zur Zeit t und η die Viskosität.

Bei nicht zu hoher Strömungsgeschwindigkeit können wir in erster Annäherung sagen, daß P_1 für jedes bestimmte Versuchsmaterial und für jede bestimmte Kapillare konstant ist, obgleich — wie später gezeigt werden wird — die Annahme von Rideal⁴⁾, derzufolge die Oberflächenspannung bei hohen Strömungsgeschwindigkeiten mit der Strömungsgeschwindigkeit variiert, nicht vernachlässigt werden darf.

¹⁾ Übersetzt von A. Siehr (Leipzig).

²⁾ G. W. Scott Blair, J. Physic. Chem. 39, 12 (1935).

³⁾ E. C. Bingham und H. A. Murray, Trans. Amer. Soc. Test. Materials 23, 655 (1923).

⁴⁾ [Maximalwert.]

⁵⁾ E. Rideal, Philos. Mag. 44, 1152 (1922).

Gleichung (2) kann folgendermaßen geschrieben werden:

$$t = - \frac{8\eta}{13,55 \text{ g} \cdot P_1 R^3} \int l \, dl$$

$$= - \frac{4\eta}{13,55 \text{ g} \cdot P_1 R^3} l^2 + C$$

(C = Integrationskonstante).

Wenn nun $l = L$ ist, so erhalten wir $t = 0$,

$$C = \frac{4\eta}{P_1 R^3} L^2$$

und daraus

$$\eta = \frac{R^3 P_1 t \cdot 13,55 \text{ g}}{4 (L^2 - l^2)}$$

oder

$$\eta = \frac{R^3 (P - P_1) t \cdot 13,55 \text{ g}}{4 (L^2 - l^2)} \quad (3)$$

Ist h die Höhe, bis zu der die Flüssigkeit bei vertikaler Stellung der Kapillare steigt, dann können wir schreiben:

$$\sigma = \frac{h R \varrho g}{2},$$

wobei ϱ die Dichte der Flüssigkeit ist.

Folglich ist

$$P_1 = \frac{2 h \varrho}{13,55} \quad (4)$$

Eine andere Methode zur Berechnung von P_1 besteht darin, daß man den Versuch bei verschiedenen Drucken durchführt und dann berechnet, welchen Wert für P_1 man in Gleichung (3) von P abziehen muß, um einen vom Druck unabhängigen Wert für η zu erhalten. Für höhere Geschwindigkeiten, bei denen P_1 nicht konstant ist, läßt sich nur die letztere Methode anwenden.

Beschreibung des Apparates.

Der in Fig. 1 wiedergegebene Apparat besteht im wesentlichen aus einer T-förmigen Glasröhre, die mit einem weiten und weichen Gummischlauch verbunden ist, der zwischen den beiden Gummirollen einer von Hand betriebenen kleinen Mangel durchgeführt ist⁵⁾. Außerdem ist das T-Stück noch mit einem Quecksilbermanometer (2) und mit einem langen engen Rohr (3) verbunden, das am anderen Ende kugelförmig erweitert ist. Es werden mehrere solcher Röhren von verschiedenem Durchmesser benutzt. An der Verbindungsstelle zwischen T-Stück und Kapillare liegt der Nullpunkt der Skala, die die Werte von $L^2 - l^2$ ($= 256 - l^2$) anzeigt. Die Röhren

werden durch Ansaugen von der Glaskugel bis zum anderen Ende mit dem Versuchsmaterial gefüllt und dann in horizontaler Lage nach oben stehen gelassen. Nach 16—17 Stunden wird ein Röhren mit dem T-Stück verbunden und ein geeigneter Druck durch schnelles Drehen der Handwinde (Zusammendrücken des Gummischlauchs) hervorgerufen. Die zweckmäßigste Höhe des Druckes kann man aus dem Verhalten der Flüssigkeiten beim Einfüllen in das Röhren schätzen oder durch einen Vorversuch mit einer zweiten Probe des Versuchsmaterials bestimmen. Die Korrektur P_1 , die höchstens 20 Proz. betragen darf, wird vorher bestimmt, und die Stellung der Skala an dem Quecksilbermanometer wird derart reguliert, daß die Ablesungen direkt den korrigierten P_2 -Werten entsprechen. Durch langsames Drehen der Handwinde wird der Druck konstant gehalten. Die Stellung des Meniskus auf der $L^2 - l^2$ -Skala wird bis zur völligen Leerung des Röhrens nach je 15 Sekunden abgelesen. Die Gesamtzeit beträgt gewöhnlich nicht weniger als 4 und nicht mehr als 10 Minuten, es sei denn, daß ein empirisch gefundener und nicht der aus der statischen Oberflächenspannung berechnete Wert für P_1 eingesetzt wurde. In letzterem Fall können Gesamtzeiten von weniger als 4 Minuten angewandt werden.

Prüfung des Apparates.

Es wurden Versuche mit Rothamsted-Honig (vorwiegend Kleeblütenhonig) durchgeführt, dessen Verhalten dem Hagen-Poiseuille'schen Gesetz gut entspricht. Diese Honigsorte wurde bei verschiedenen P -Werten untersucht, und es wurden während des Ausfließens bei jedem Druck

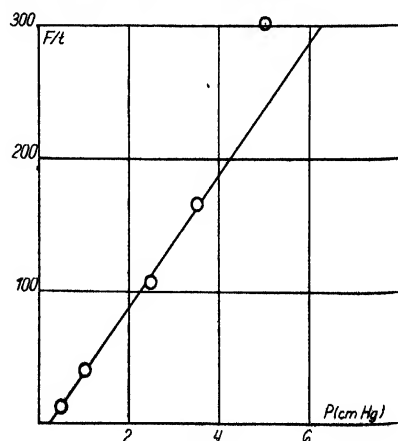


Fig. 2

⁵⁾ Der Verfasser dankt Herrn D. M. T. Morland, für die Anregung zu dieser Art von Druckanwendung.

etwa 10 Ablesungen vorgenommen. Die Durchschnittswerte von F/t jeder dieser Versuchsreihen sind in Fig. 2 gegenüber P aufgetragen.

Wie man aus Fig. 2 ersehen kann, erhält man bei einer nicht zu schnellen Strömung eine innerhalb der Fehlergrenze konstante Viskosität, wenn man für P_1 einen Wert von etwa 0,2 cm Hg wählt. Dies entspricht ungefähr dem aus der statischen Oberflächenspannung des Honigs berechneten Wert.

Zahlreiche Honigsorten wurden nach dieser Methode untersucht. Fig. 3 zeigt den charakteristischen Unterschied zwischen einem guten Honig aus *Calluna vulgaris*, der Thixotropie zeigte [A]⁶⁾, und einem wirklich flüssigen Honig, der aus anderen Blüten stammte (B).

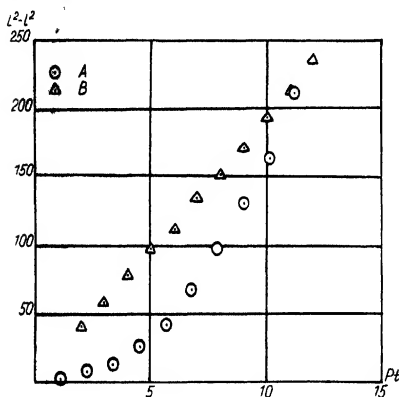


Fig. 3

Die Punkte in Fig. 4 geben die experimentell erhaltenen Daten wieder, die nach der oben beschriebenen Versuchsmethode bei einem wirklich flüssigen Honig erhalten wurden. Die Gerade entspricht der Viskosität desselben Versuchsmaterials, die durch Auspressen des Honigs bei hohem Druck aus der gefüllten Kugel durch die gleiche Röhre und durch Wägen des nach einer bestimmten Zeit ausgeflossenen Honigs bestimmt wurde. Die Dichte wurde mit Hilfe eines Pyknometers bestimmt. Die Übereinstimmung ist zufriedenstellend, und die leichte Krümmung der

Geraden, die auf eine Änderung der (dynamischen) Oberflächenspannung in Abhängigkeit von der Strömungsgeschwindigkeit zurückzuführen ist, ist nicht stark genug, um die Zuverlässigkeit der Versuchsergebnisse zu beeinträchtigen.

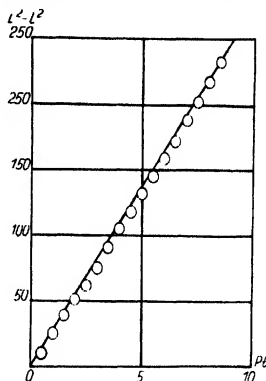


Fig. 4

Bei diesen Erörterungen wurde der Ausdruck „Thixotropie“ in dem Sinne gebraucht, daß die Konsistenz eines Stoffes unter dem Einfluß der Scherung abnimmt, bei anschließendem Stehen jedoch wieder mehr oder weniger ihren ursprünglichen Wert erreicht. Hierher gehört auch die Erscheinung, bei der man genauer von „False Body“ spricht, da hierbei keine wahre Gel-Sol-Umwandlung eintritt, und das Versuchsmaterial unter dem Einfluß der Scherung elastische Eigenschaften auch dann zeigt, wenn es noch keine Zeit hatte, seine Konsistenz zu erhöhen [vgl. Pryce-Jones⁷⁾].

Zusammenfassung.

Es wird ein Viskosimeter für sehr kleine Flüssigkeitsmengen beschrieben. Der Apparat ist besonders geeignet, um den Grad der Abweichung vom Hagen-Poiseuille'schen Gesetz bei Nicht-Newton'schen Flüssigkeiten und thixotropen Systemen zu bestimmen. Außerdem gibt er ein empirisches Maß für den Grad der Thixotropie.

* * *

Verfasser dankt Herrn Dr. R. K. Schofield für fördernde Kritik und Ratschläge bei der Niederschrift dieser Arbeit.

⁷⁾ J. Pryce-Jones, J. Oil and Colour Chem. Assoc. 17, 305 (1934).

⁶⁾ In den meisten Fällen erhält man für thixotrope Honigsorten beim Auftragen von $\log(Pt)/\log F$ eine Gerade. Die Neigung dieser Geraden kann als empirisches Maß für die Abweichung vom Hagen-Poiseuille'schen Gesetz betrachtet werden, jedoch kommt dieser Methode keine theoretische Bedeutung zu.

Bemerkung zum Mechanismus der Spinnbarkeit.

Von R. K. Schofield und G. W. Scott Blair.

(Aus der Physikalischen Abteilung der Rothamsted Experimental Station Harpenden, England.)

(Eingegangen am 22. Februar 1937.)

Die Eigenschaft der „Spinnbarkeit“, das ist die **Neigung** einer Flüssigkeit, sich zu Fäden ausziehen zu lassen, ist von mehreren Forschern, insbesondere von H. Erbring untersucht worden. Dieser Autor hat gezeigt¹⁾, daß diese Eigenschaft weder mit der Viskosität noch mit der gewöhnlichen (statischen) Oberflächenspannung in direktem Zusammenhang steht, und wegen der großen Zahl von Stoffen, bei denen er Spinnbarkeit fand, wäre es von Interesse, Näheres über den eigentlichen Mechanismus zu erfahren.

Gewisse südafrikanische Honigarten sind als „fadenziehend“ („stringy“) beschrieben worden. Durch die Freundlichkeit von Herrn J. Pryce Jones waren die Autoren in der Lage, eine Probe eines solchen Honigs zu untersuchen. Er erwies sich bei der Prüfung auf Spinnbarkeit in der Tat als besonders stark fadenziehend. So konnten z. B. bei einer Spinn geschwindigkeit von 5 cm/Sek. Fäden von ca. 75 cm Länge erhalten werden²⁾.

Wir haben die Probe weiterhin in dem kürzlich beschriebenen Thixotrometer untersucht³⁾ und eine annähernd geradlinige Kurve erhalten. Die Substanz fließt mit einem eigenartig gekrümmten Meniskus von der in Fig. 1 dargestellten

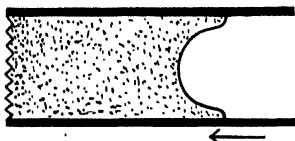


Fig. 1

Form. Unter Berücksichtigung der Ungenauigkeiten, die beim Ablesen der Stellung eines solchen Meniskus entstehen, scheint unter den vorliegenden Versuchsbedingungen jedoch keine nennenswerte Abweichung vom Hagen-Poiseuille'schen Gesetz aufzutreten. Die Oberflächenspannung ist nicht anormal, auch wird sie nicht schätzbar beeinflusst durch Veränderung der Größe der untersuchten Oberfläche.

¹⁾ H. Erbring, Kolloid-Beih. 44, 171 (1936); Kolloid-Z. 77, 32 (1936); 77, 213 (1936).

²⁾ Die Verfasser danken Herrn Dr. Erbring für seine Mithilfe und für die Durchführung dieser Versuche sowie für die Übersetzung dieser Arbeit.

³⁾ G. W. Scott Blair, Kolloid-Z. 78, 19 (1937).

Unsere kürzlichen Versuche an Mehlteig⁴⁾ haben unser Interesse auf die Erscheinung der „Streckhärtung“ geführt („work-hardening“), und der folgende einfache Versuch zeigte, daß, wenigstens im Falle des hier in Frage kommenden Honigs, die Spinnbarkeit auf das Vorhandensein einer elastischen Netzstruktur innerhalb des Stoffes zurückzuführen ist. Wir müssen annehmen, daß diese Netzstruktur zu schwach ist, um selbst im Thixotrometer festgestellt werden zu können, wo verhältnismäßig große Kräfte angewandt werden, so daß der Stoff sich fast wie eine echte Flüssigkeit verhält. Ein Honigtropfen wurde auf eine reine Quecksilberoberfläche gebracht und zwei kleine Korkstückchen an den gegenüberliegenden Seiten des Tropfens befestigt. Das eine Stück wurde starr befestigt und das andere konnte durch Ziehen eines an ihm angehefteten Fadens bewegt werden. Wenn der Tropfen ausgedehnt wurde, so daß er einen länglichen stäbchenförmigen Körper bildete, und wenn, nachdem er einige Minuten unter Spannung gehalten worden war, diese aufgehoben wurde, so pflegte er langsam seine ursprüngliche Gestalt wieder einzunehmen und verhielt sich also fast wie ein rein elastischer Stoff. Ähnliche Versuche an „nichtfadenziehenden“ Honigarten und wässrigen Zuckersirupen zeigten ebenfalls eine gewisse elastische Rückbewegung, aber bei diesen letzteren Stoffen neigten die durch Streckung erzeugten stäbchenförmigen Körper dazu, eine Einschnürung zu bilden und sehr schnell zu reißen, besaßen also viel weniger ausgesprochen elastische Eigenschaften.

Es ist klar, daß ebenso wie bei den Mehlteigen auch in dem vorliegenden „fadenziehenden“ Honig eine elastische Netzstruktur vorhanden sein muß. Diese bewirkt vermutlich das Auftreten von „Spinnbarkeit“ ebenso wie die abnormen Menisken, wenn die Substanz durch ein Rohr fließt. Die Frage, ob eine solche Struktur auch bei anderen spinnbaren Flüssigkeiten besteht, sollte untersucht werden.

⁴⁾ R. K. Schofield und G. W. Scott Blair, Proc. Roy. Soc. London, Ser. A 138, 707 (1932); 139, 557 (1933); 141, 72 (1933), ein weiterer Teil im Druck; Kolloid-Z. 79, 148 (1937).

179. *A New Calcium Silicophosphate.*

By GUNTER NAGELSCHMIDT.

IN the course of a mineralogical investigation of a typical series of modern basic slags which had been tested as phosphatic fertilisers in field and pot culture experiments (Crowther, *J. Roy. Agric. Soc.*, 1934, 95, 1), a new material was found as a major constituent of some open-hearth slags with citric acid solubilities between 40 and 80%. Although at least 80% of the material in commercially ground basic slags is guaranteed to pass through the British Standard Test Sieve, mesh number 100, a few individual particles may be as large as 0.5 mm. in diameter and a small fraction is below the range of microscopic visibility. Most of the grains are heterogeneous; they contain inclusions, different materials are intergrown, and it is therefore very difficult to isolate the pure components, although these can often be recognised and identified by microscopical and microchemical methods. In the present work it was possible to isolate the new compound in three stages.

(1) A suitable grain size group of about 0.2–0.02 mm. diameter was separated by sieving and sedimentation in water saturated with calcium carbonate. (2) A magnetic separation was carried out by using Hallimond's separator (*Min. Mag.*, 1930, 22, 377),* a non-magnetic fraction being obtained in which all the grains were more or less free from dark inclusions. (3) A gravity separation was effected by repeated centrifuging with methylene iodide-benzene mixtures. The new compound was found to have d^{20}_D 3.035 ± 0.005 .

Optical Properties.—The material is colourless, and biaxial positive. The angle of the optical axes is very small. The largest and the smallest refractive indices observed with sodium light are n_x , 1.661 and n_z , 1.652 ± 0.002 . The grains frequently have two opposite parallel edges with straight extinction along these edges. No other crystallographic boundaries occur, and the crystal symmetry is unknown, but cannot be higher than orthorhombic.

Chemical Composition.—From 25 g. of slag the above method of separation afforded a 0.5 g. fraction which seemed to be sufficiently pure for chemical analysis, although it still contained a small percentage of foreign materials. Analyses made on 127 and 234 mg. gave the results shown

* The author is indebted to Prof. P. G. H. Boswell for permitting him to use this apparatus at the School of Mines of the Imperial College of Science and Technology.

in Table I, (i) and (ii) respectively, which give $\text{CaO}:\text{P}_2\text{O}_5:\text{SiO}_2 = \text{approx. } 7:1:2$. If a recalculation is made on this basis it appears that the analysed material contained 92% of this compound, contaminated with tricalcium silicate and some spinelloid material. In view of this contamination and the fact that the analyses account only for 97% of the material, the formula of the new compound cannot be established with certainty, but $7\text{CaO}, \text{P}_2\text{O}_5, 2\text{SiO}_2$ seems the most probable.

Körber and Trömel (*Arch. Eisenhüttenw.*, 1933, 7, 7), investigating the ternary system $\text{CaO}-\text{P}_2\text{O}_5-\text{SiO}_2$, found two ternary compounds. The first had the composition $5\text{CaO}, \text{SiO}_2, \text{P}_2\text{O}_5$ and was identical with silicocarnotite, which has long been known as the main constituent of basic Bessemer slags. To the second compound they ascribed the composition $9\text{CaO}, \text{P}_2\text{O}_5, 8\text{SiO}_2$, but later (Trömel, private communication), $8\text{CaO}, \text{P}_2\text{O}_5, 2.5\text{SiO}_2$ was found to agree better with the data. It is not yet known, however, how far this second compound is able to form mixed crystals with the other components of the ternary system, and further synthetic work will be needed to elucidate this point. Both ternary compounds had citric acid solubilities above 95%.

The author is much indebted to Dr. Trömel for supplying him with a sample of his second ternary compound, which proved to have very similar optical properties to those described for the material $7\text{CaO}, \text{P}_2\text{O}_5, 2\text{SiO}_2$ isolated as detailed above. X-Ray powder diagrams * of the two materials proved to be identical, both in the position and in the intensities of the lines. The data are in Table II, together with comparable data for silicocarnotite, which gave a different pattern. This proves that the new compound is not a mixed crystal of silicocarnotite and dicalcium silicate. In view of the low crystal symmetry, the complex chemical composition, and the absence of suitably formed single crystals, no attempt was made to determine the crystal structure of the new material from the X-ray data.

TABLE I.
Chemical analysis of new compound.

	(i).	(ii).	Calc. for $7\text{CaO}, 2\text{SiO}_2, \text{P}_2\text{O}_5$.
CaO, %	58.3	58.4	60.0
P_2O_5 , %	19.9	20.0	21.6
SiO_2 , %	17.9	17.7	18.4
$\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3$, %	—	0.4	—
MgO, %	—	0.6	—
Total, %	96.1	97.1	100.0

TABLE II.

Powder diagrams of silicocarnotite (I), the new compound (II), and Trömel's synthetic material (III), taken with copper K- α radiation; interplanar spacings (d) and intensities (I) are recorded.

	(I).		(II).		(III).	
No.	<i>d.</i>	<i>I.</i>	<i>d.</i>	<i>I.</i>	<i>d.</i>	<i>I.</i>
1	4.58	w.	3.93	w.	3.93	w.
2	3.96	m.	3.51	w.	3.52	w.
3	3.60	v.w.	2.85	s.	2.86	s.
4	3.33	m.	2.70	s.	2.70	s.
5	2.99	m.	2.33	v.w.	2.33	v.w.
6	2.83	v.s.	2.22	v.w.	2.24	v.w.
7	2.61	v.s.	1.960	s.	1.961	s.
8	2.29	m.	1.862	v.w.	1.862	w.
9	2.18	m.	1.761	m.	1.761	m.
10	2.03	w.	1.668	w.	1.666	w.
11	1.958	w.	1.591	w.	1.590	w.
12	1.880	s.	1.562	w.	1.562	w.
13	1.812	w.	1.485	m.	1.487	m.
14	1.750	m.	1.346	m.	1.347	m.
15	1.691	m.	1.260	v.w.	1.262	v.w.
16	1.644	v.w.	1.220	v.w.	1.219	v.w.
17	1.538	w.	1.185	v.w.	1.185	v.w.
18	1.510	w.				
19	1.241	w.				
20	1.159	w.				
21	1.136	w.				
22	1.046	v.w.				
23	1.030	v.w.				

v.s. = very strong, s. = strong, m. = medium, w. = weak, v.w. = very weak.

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SUMMARY.

A new compound has been isolated from certain medium-soluble open-hearth basic slags, with a chemical composition approximating to $7\text{CaO}, \text{P}_2\text{O}_5, 2\text{SiO}_2$. It has d^{20} 3.035, n_γ 1.661, n_α 1.652 for sodium light, and is colourless and biaxial positive with a very small axial angle. X-Ray powder data are given. This material is almost identical with a synthetic calcium silicophosphate of approximate composition $8\text{CaO}, \text{P}_2\text{O}_5, 2.5\text{SiO}_2$ prepared by Trömel.

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